HEMATOPOIESIS

Organizers: David W. Golde and Steven C. Clark

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Hematopoiesis

Keynote Address

H 001 HEMATOPOIETIC STEM CELLS AND EARLY HEMATOLYMPHOID DIFFERENTIATION IN MOUSE/MAN, I. Weissman, S. Heimfeld, G. Spangrude, R. Namikawa, M. Lieberman, H. Kaneshima, L. Smithy, C. Guidos, B. Adkins and M. McCune, Stanford University School of Medicine, Stanford, CA 94305

The study of mammalian hematolymphopoiesis offers to the developmental biologist at the same time a system that allows precise analysis of <u>in vitro</u> models and <u>in vivo</u> outcomes, as well as the possibility of rapid transfer of obtained knowledge to contemporary clinical problems. Using antibodies directed against hematolymphoid lineage markers with various cell-sorting strategies for the adoptive transfer of cells between congenic mouse strains we have recently isolated 1) the pluripotent hematopoietic stem cell; 2) a multipotent progenitor that apparently does not self-renew; and 3) several lineage specific progenitors.

In order to study parallel developmental processes in human hematolymphoid differentiation, and especially to provide models to study the pathogenesis of human hematotropic and lymphotropic viruses (such as HIV), we have established a working model of human lymphopoiesis in immunodeficient (SCID) mice. Implantation of human fetal thymus under the SCID mouse kidney capsule allows full development of thymocytes and peripheral T cells from i.v. injected human fetal liver hematopoietic cells. Implantation of human fetal lymph nodes (and/or spleen) allows reconstitution of lymphoid architecture, and the differentiation of human B cells to antibody secreting plasma cells. Preliminary studies indicate that these hosts will prove useful in the identification of human hematolymphoid progenitors, and to study the <u>in vivo</u> pathogenetic potential of molecularly cloned HIV.

Growth Factors/Structure Function

H 002 **The Hematopoietic Activity of GM-CSF is Dependent upon Two Distinct Regions:A Functional Analysis Based upon the Activities of a Series of Human-Mouse Hybrid Growth Factors and Neutralizing Monoclonal Antibodies** Kenneth Kaushansky Div. of Hematology, Univ. of Washington Seattle, WA.

GM-CSF is an acidic glycoprotein required for the proliferation and differentiation of hematopoietic progenitors, and for the activation of mature blood cells. Despite a high degree of homology, human (h) and murine (m) GM-CSF fail to cross-react in functional assays. Based upon this finding, a strategy was developed to determine the sequences required for species-specific function, and hence, the sequences required for receptor binding. Using fragments of h and m GM-CSF cDNA, a series of chimeric hm GM-CSF expression vectors were developed and the resultant hybrid growth factors analyzed. As deduced from the data below, two regions of GM-CSF are required for both progenitor and mature blood cell function.

hm hybrid map	Activities: Progenitor h	Progenitor m	Mature Cell h	Receptor Comp h	Receptor Comp m
h 31 m	-	+++	-	-	+
h 37 m	-	++	-	-	+
h 48 m	-	-	-	-	-
h 110 m	+++	-	+++	+++	-
h 92 m	+	-	+	±	-
h 78 m	-	-	-	-	-
m 37 h 56 m	-	-	-	-	-
m 92 h 110 m	-	-	-	-	-
m 37 h 110 m	+++	-	+++	+++	-

The first region, an amphiphilic alpha helix, is located between Glu_{38} and Asp_{48} (1=Met). The second, a disulfide stabilized loop, is located between Pro_{93} and Glu_{110}. Neither region alone could stimulate species-specific function, and neither could displace ^{125}I GM-CSF from its receptor. Finally, neutralizing monoclonal antibodies were found to bind to each of these regions. These studies provide a basis for the development of altered cytokine molecules which might have advantageous clinical properties.

H 003 GROWTH FACTORS REGULATING HEMOPOIETIC STEM CELLS, Makio Ogawa, VA Medical Center and Department of Medicine, Medical University of SC, Charleston, SC 29403

The central feature of hemopoiesis is the life-long, stable cell renewal. This process is supported by hemopoietic stem cells which, in the steady-state, appear to be dormant in cell cycling and reside in the so-called G_0 state. The entry into cell cycle of the dormant stem cells may be promoted by such factors as interleukin-1 (IL-1), interleukin-6 (IL-6) and granulocyte colony-stimulating factor (G-CSF). Available evidence indicate that the effects of IL-1 on the dormant stem cells is indirect and in part mediated by IL-6 and G-CSF. Once the stem cells leave the G_0 and begin proliferation, the subsequent process is characterized by continued proliferation and differentiation. While several models of stem cell differentiation have been proposed, our micromanipulation studies of individual progenitors suggest that the commitment of multipotential progenitors to single lineages is a stochastic (random) process. The proliferation of early hemopoietic progenitors requires the presence of lineage nonspecific factors including interleukin-3, interleukin-4 and granulocyte/macrophage colony-stimulating factor (GM-CSF). Once the progenitors are committed to individual lineages, the subsequent maturation process appears to be supported by late-acting, lineage-specific factors such as erythropoietin (Ep), monocyte/macrophage colony-stimulating factor (M-CSF), G-CSF or interleukin-5 (IL-5). Ep is the physiological regulator of erythropoiesis. While the physiological roles of other factors have not been elucidated, the targets of M-CSF, G-CSF and IL-5 are thought to be late stages of monocyte, neutrophil and eosinophil development, respectively.

EPO/Megakaryopoiesis

H 004 CHARACTERIZATION OF ERYTHROPOIETIN RECEPTORS, Sanford B. Krantz, Stephen T. Sawyer, Ken-Ichi Sawada, and Takayuki Hosoi, Dept. of Medicine, Vanderbilt University School of Medicine and Veterans Administration Med. Center, Nashville, TN 37232

Study of erythropoietin (Epo) receptors on colony-forming units-erythroid (CFU-E) as required purification of these cells since they are present in the bone marrow in very low frequency. In the murine system, the Friend virus that produces anemia (FVA) has been used to generate highly purified CFU-E in the spleens of mice infected with the virus. Using these cells and ^{125}I-recombinant human Epo (r-HuEpo) we have found 950 total receptors on the cell surface including 300 with a K_d of 0.09 nM, while the remaining binding sites had a K_d of 0.57 nM. Cross-linking of ^{125}I-rHuEpo to FVA-cells showed proteins of 100 kDa and 85 kDa. The relatedness of these two receptor proteins was analyzed by proteolytic mapping using the Cleveland method and by digestion of cross-linked membranes with mixtures of glycanases which removed N-linked, O-linked sugars, and sialic acid. Identical lower molecular weight fragments were generated by protease treatment of the 140 kDa and 125 kDa ^{125}I-rHuEpo cross-linked receptor complex and both bands appeared to lack detectable glycosylation suggesting that the two proteins of the Epo receptor may be the same gene product, but processed differently. Only lower affinity receptors for r-HuEpo were detected on CFU-E purified from the spleens of mice infected with the polycythemia strain of Friend virus and a murine erythroleukemia cell line, both of which are not responsive to rHuEpo in culture. Internalization of ^{125}I-rHuEpo occurred in all of the above cells at about the same rate. Placentas from mice and rats were found to have only lower affinity receptors for rHuEpo and when membranes were prepared from all of the above cells as well as from mice and rat placentas, cross-linking with ^{125}I-rHuEpo revealed the same 100 kDa and 85 kDa bands.

We have also developed a method for preparing highly purified human CFU-E and have demonstrated that these cells have the capacity to bind ^{125}I-rHuEpo. Quantitation of Epo receptors on these cells revealed a total of 1050 binding sites per CFU-E of which 1/5 had a Kd of 0.10 nM while the remainder had a Kd of 0.57 nM. Internalization of ^{125}I-rHuEpo was similar to the above cells. Intact human CFU-E were used to identify the receptor for rHuEpo by cross-linking of ^{125}I-rHuEpo and this revealed two similar bands of a 100 kDa and 90 kDa. Thus, human and mouse CFU-E have a similar number of Epo receptors of high and lower affinity with similar dissociation constants and a similar gross structure. Non-responsive erythroid cells and placenta have only the lower affinity receptors and only cells with high affinity receptors respond to r-HuEpo with erythroid maturation.

H 005 CYTOKINES WHICH INFLUENCE MEGAKARYOCYTOPOIESIS. D.E. Williams, P.J. Morrissey, D. Krumweih, D.L. Urdal and S. Gillis, Departments of Cellular Biochemistry and Immunology, Immunex Corp., Seattle, WA 98101 and Biochemistry and Immunology, Behringwerke AG, Marburg FRG.

Megakaryocytes are the cellular source of circulating platelets and arise from a process of proliferation and differentiation of immature progenitor cells (CFU-MK) followed by maturation events in the nucleus and cytoplasm of morphologically recognizable megakaryocytes. These processes are thought to be regulated by different cytokines; a megakaryocyte-CSF (MK-CSF) and a maturation factor (MMF). Of the currently available cytokines, IL-3, GM-CSF, G-CSF, IL-4 and EPO have all been shown to have *in vitro* MK-CSF activity while IL-3, GM-CSF, EPO and a factor from human embryonic kidney cells influence maturation. In the current studies, the known cytokines were assessed for their influence on megakaryocyte development *in vivo* using cynomologus monkeys and/or mice. In normal monkeys, modulation of circulating platelets was achieved with the combination of IL-3 followed by GM-CSF, but not by either cytokine alone. This suggests that IL-3 primes the platelet producing system to respond to GM-CSF. In normal mice, IL-1β which acts on primitive cells and is thought to both induce CSF receptors and stimulate CSF production, gave a dose dependent increase in the number of bone marrow and splenic CFU-MK, and spleen megakaryocytes. Despite this megakaryocytic expansion, platelet counts were transiently depressed by 30-35%. IL-1β treated mice may be a useful animal model system to investigate factors which result in enhanced platelet production.

Receptors/Mechanism

H 006 HUMAN GM-CSF RECEPTORS AND TARGET CELL INTERACTIONS, Judith C. Gasson, Gayle C. Baldwin, James S. Economou, Adi Gazdar, David W. Golde, and John F. DiPersio, Division of Hematology-Oncology, Department of Medicine; Division of Surgical Oncology, Department of Surgery, UCLA School of Medicine, Los Angeles, CA 90024-1678; Medical Oncology Branch, National Cancer Institute and National Naval Medical Center, Bethesda, MD 20014.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates the proliferation of bone marrow-derived myeloid progenitor cells, as well as fresh myeloid leukemic cells and established cell lines. In addition, GM-CSF enhances multiple differentiated functions of mature neutrophils, monocytes, and eosinophils. These diverse biological activities are consistent with crucial physiological roles for GM-CSF in hematopoiesis and host defense, as well as potential pathophysiological roles in leukemias and inflammatory disorders. All of the biological activites of GM-CSF appear to be mediated by binding of GM-CSF to a single class of high-affinity (Kd=30 pM) receptors present in low numbers ($\leq$1,000 sites/cell) on all responsive cell types. Cross-linking studies reveal a single protein of an apparent molecular weight of 84,000 daltons, which binds GM-CSF in all cells and cell lines tested. Recently, we have demonstrated that the presence of GM-CSF receptors is not limited to hematopoietic cells. Equilibrium binding and cross-linking studies demonstrate the presence of GM-CSF receptors on small cell carcinoma of the lung cell lines. These studies have been expanded to characterize the expression of GM-CSF receptors on cell lines from other types of neuroendocrine tumors. In addition, we have shown that fresh surgical neuroendocrine tumor samples have receptors for GM-CSF. These observations have important implications in terms of potential physiological functions of GM-CSF, as well as therapeutic uses to ameliorate chemotherapy-induced bone marrow suppression in solid tumor patients.

The mechanism(s) by which GM-CSF exerts its multiple biological effects are not well understood. We compared the pattern of gene expression induced by GM-CSF in proliferating and terminally differentiated cells. A family of primary response genes known as TIS sequences (TPA-inducible sequences; Lim et al., *Oncogene* 1:263, 1987) was used to screen mRNA prepared from a GM-CSF-treated factor-dependent murine cell line and human neutrophils. The results show a rapid and unique pattern of induction of TIS gene expression common to both cell types. Studies are underway to characterize the mechanism by which GM-CSF induces expression of primary response genes in diverse cell types.

H 007 CSF-1 AND ITS RECEPTOR, Charles J. Sherr, Howard Hughes Medical Institute, Dept. of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN 38104

The mononuclear phagocyte colony stimulating factor, CSF-1 or M-CSF, induces the proliferation and supports the survival of monocytes, macrophages, and their committed bone marrow progenitors. The pleiotropic effects of the growth factor are mediated through its binding to a single class of high affinity receptors encoded by the c-fms proto-oncogene. The CSF-1 receptor (CSF-1R) is an integral transmembrane glycoprotein consisting of an extracellular ligand-binding domain, a single membrane-spanning segment, and an intracellular tyrosine kinase domain. Binding of CSF-1 to its receptor induces a conformational change which activates the receptor tyrosine kinase, leading to a cascade of biochemical events that culminate in mitogenesis. Transduction of the human c-fms gene to mouse NIH-3T3 fibroblasts or to interleukin-3 dependent FDC-P1 myeloid cells enables them to respond mitogenically to human recombinant CSF-1, demonstrating that receptor "switching" can reprogram a cell's ability to respond to environmental signals for growth and/or survival.

The latent transforming potential of the human c-fms proto-oncogene product can be activated by a point mutation in the CSF-1R extracellular domain, enabling the receptor to function constitutively as a tyrosine kinase and provide persistent signals for cell growth in the absence of CSF-1. A second mutation at the receptor C-terminus further up-regulates its mitogenic activity but is insufficient in itself to induce transformation. The activating mutation does not disrupt the CSF-1 binding site, indicating that it induces a conformational change that mimics the effects of ligand.

The CSF-1R and platelet-derived growth factor receptor (PDGF-R) genes map in a tandem array on the long arm of human chromosome 5. A 5' untranslated exon of the c-fms gene is located less than 0.5 kb from the 3' end of the PDGF-R gene, and these intervening sequences appear to lack promoter activity. The localization and organization of the CSF-1R and PDGF-R genes therefore suggest that they arose through duplication and may be subject to coregulation by processes involving long range splicing.

Cellular Interactions

H 008 CELL INTERACTIONS AND GENE EXPRESSION IN HAEMOPOIETIC PROGENITOR CELLS MF Greaves, MY Gordon, HV Molgaard and AM Ford, Leukaemia Research Fund Centre, Institute of Cancer Research, Fulham Road, London SW3, England.

A primitive progenitor cell with self-renewal capacity has been identified in human bone marrow that forms blast cell colonies when in contact with stromal cells (1). These progenitors express 'docking' or adhesion receptors for matrix sites which are absent or cryptic on descendent CFU-Mix and CFU-GM. The latter cells, as well as T and B cell precursors, may have their own selective docking sites. The stroma-dependent proliferative response of blast progenitor cells is likely to be regulated by locally produced growth factors that are themselves matrix associated (2).

Blast progenitor cells, as well as other lineage-restricted progenitors, express a unique marker glycoprotein - CD34 which is absent from differentiating progeny (3). Structural characteristics of CD34 protein, chromosome mapping of the human CD34 gene and molecular cloning of the murine equivalent will be described.

Interactions between multipotential stem cells and specific stromal sites may influence or dictate lineage commitment. To investigate this possibility, we have analysed chromatin configuration and expression of several lineage specific genes that are known to be expressed early in precursor cells (4). Normal multipotential IL3-dependent murine cells have accessible, DNAse I hypersensitive IgH enhancer and CD3 enhancer sites and actively transcribe unrearranged T cell receptor γ genes. When these cells are induced by stromal contact to undergo myeloid differentiation, the lymphoid receptor genes are closed down. This observation has interesting implications for lineage specification and for the cellular phenotypes observed in stem cell leukaemias.

1. Gordon MY, Dowding CR, Riley GP & Greaves MF (1987) J Cell Physiol 130:150
2. Gordon MY, Riley GP, Watt SM & Greaves MF (1987) Nature 326:403
3. Watt SM, Karhi K, Gatter K, Furley AJW, Katz FE, Healy LE, Altass LJ, Bradley NJ, Sutherland DR, Levinsky R & Greaves MF (1987) Leuk 1:417
4. Ford AM, Watt SM, Furley AJW, Molgaard HV & Greaves MF (1988) EMBO J 7: 2393

Gene Expression

H 009 IL-1 INDUCES GM-CSF mRNA ACCUMULATION IN HUMAN STROMAL CELLS (HSC) BY INDUCING RIBONUCLEASE INHIBITORY ACTIVITY. Grover C. Bagby, Gray Shaw, Melissa.A. Brown, Steven Hefeneider, Michael H. Heinrich, Louise Band, Gerald M. Segal. The Molecular Hematology/Oncology Laboratory, VA Medical Center, Portland OR 97201, Division of Hematology & Medical Oncology, Oregon Health Sciences University, Portland, OR 97201, and Genetics Institute, Cambridge, MA 02140.

GM-CSF shares a conserved motif in the 3' untranslated region with a variety of interleukins and colony stimulating factors. Because this motif accounts for high rates of transcript decay, and because IL-1 induces GM-CSF, G-CSF and IL-6 transcript accumulation, we tested the hypothesis that IL-1 effects these changes by inhibiting mRNA degradation rate which is, because of a 51 bp element in the 3' untranslated region (3'UT), ordinarily high. To examine the effects of IL-1 on GM-CSF and IL-6 mRNA stability, untreated and IL-1 stimulated cells were exposed to Actinomycin D for various periods of time, poly A+ RNA was harvested and GM-CSF transcripts measured by Northern analysis. The T½s of GM-CSF and IL-6 mRNA in uninduced cells was <3 hr, but in IL-1 induced cells were >24 hr. In order to determine the mechanism by which IL-1 confers such extreme stability to mRNA, we used a modification of an in vitro degradation technique described by Ross et. al. (J. Biol. Chem, 1987) in which polysome bound ribonucleases (pbr) are incubated with uniformly labeled in vitro RNA transcripts and the effect of pbr on RNA stability is measured over time by PAGE. Full length, 5' capped and polyadenylated transcripts of GM-CSF cDNA and δGM-CSF cDNA (from which the 3'UT motif had been deleted) were exposed to pbr from induced and uninduced cells. Both mRNAs were completely degraded within 20 min by pbr from uninduced cells, but were readily detectable even after 40 min of incubation with pbr from IL-1-induced cells. A cytosol extract (C) from induced cells inhibited degradation mediated by uninduced pbr but C from uninduced cells did not. Therefore, GM-CSF and IL-6 are constitutively transcribed in stromal cells and transcripts are briskly degraded in the steady state. IL-1 regulates transcript accumulation, at least in part, by inducing expression of an inhibitor of ribonuclease(s), and this regulation may be independent of the 3'UT.

H 010 REGULATION OF GM-CSF EXPRESSION, Stephen D. Nimer, John Fraser, Irvin S.Y. Chen, H. Phillip Koeffler, Joseph D. Rosenblatt, Judith C. Gasson, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024-1678

Human granulocyte-macrophage colony-stimulating factor (GM-CSF) has potent in vitro and in vivo effects on hematopoiesis, and on the function of mature myeloid effector cells. The expression of GM-CSF is tightly regulated by a series of transcriptional and post-transcriptional mechanisms. Accumulation of GM-CSF mRNA or secretion of GM-CSF protein by resting normal cells has not been demonstrated, but a limited number of normal cellular sources (T lymphoblasts, fibroblasts, endothelial cells, and mononuclear phagocytes) produce GM-CSF in response to cytokine or chemical stimulation. T-cell activation, whether by mitogen, lectin or antigen, induces expression of a variety of lymphokines, including GM-CSF. To identify the DNA sequences that convey the T-cell activation signal to the GM-CSF gene, we performed transient transfection assays introducing recombinant GM-CSF-CAT constructs, into a variety of T-cell lines and into primary human T cells. Using this approach, we have identified regulatory regions contained within the GM-CSF 5' flanking sequences that either positively or negatively affect transcription of the GM-CSF gene. We have focused our recent efforts on a 30-bp region in the GM-CSF promoter (-53 to -24), which contains sequences essential for basal and inducible T-cell activity. Contained within this region is the repeated sequence CATT(A/T), and our deletion mutant and site-directed mutagenesis studies demonstrate that these CATT repeats are required for GM-CSF promoter activity. Prior DNase I footprinting experiments demonstrate binding of nuclear proteins to a 34-bp region that contains these CATT(A/T) repeats and a portion of the TATA homology. The effects of mutations in this 34-bp region on binding of nuclear proteins is being investigated.

Unlike resting T cells, HTLV-infected T-cell lines constitutively express GM-CSF. To explore the molecular mechanisms involved in this constitutive expression, we performed co-transfection studies in a variety of cell lines using HTLV tax-expressing constructs and GM-CSF promoter CAT constructs. Our results show that the trans-activating tax protein of HTLV-I and -II can activate GM-CSF-CAT constructs via sequences located just upstream of the GM-CSF initiation site. The sequences involved and the cell specificity of this activation will be discussed. We will also present data on the expression of GM-CSF by fibroblasts which demonstrate that different mechanisms are utilized by fibroblasts to produce GM-CSF in response to tumor necrosis factor, interleukin-1 and phorbol diesters.

Hematopoiesis

Stem Cells

H 011 ONTOGENY OF MULTIPOTENT CD7+ T CELL PRECURSORS: A MODEL FOR THE INITIAL STAGES OF HUMAN T CELL DEVELOPMENT, Barton F. Haynes, Stephen M. Denning, Kay H. Singer and Joanne Kurtzberg, Departments of Medicine and Pediatrics, Duke University Medical Center, Durham, North Carolina, 27710.

Understanding the earliest stages of T cell development is important for study of immunodeficiency diseases and for developing effective methods of immune reconstitution. In addition, understanding the genesis of autoimmunity necessitates knowing how cells of the thymic microenvironment interact with immature T cell precursors. We have used monoclonal antibodies that react with T cell precursors prior to their entry into the thymic rudiment, coupled with progenitor assays to define and characterize cells capable of differentiation to the T lineage. Moreover, we have established in vitro assays of thymocyte-thymic epithelial (TE) cell interactions. We have used the CD7 monoclonal antibody to isolate CD7+ cells from first trimester fetal tissues and postnatal thymus, and as well to study CD7+, CD4-, CD8-, CD3- (triple negative) leukemic cells. CD7+ triple negative normal and malignant cells upon stimulation with PHA-conditioned media and IL2 gave rise to mature T cells. Interestingly, both normal and leukemic CD7+ triple negative cells, when cultured under conditions favorable for myeloid differentiation, gave rise to CFU-GEMM and CFU-GM colonies as well. The presence of 1,14 or 7,14 chromosome translocations in two of the CD7+ leukemic suspensions allowed for a precursor-product relationship to be determined, demonstrating that leukemic CD7+ triple negative cells were indeed multipotent. These data have given rise to a model pathway of early T cell maturation whereby CD7+ multipotent T cell precursors (pro-T cells) colonize the human thymus and give rise to committed pre-T cells which in turn give rise to T cells expressing either $\alpha\beta$ or $\gamma\delta$ T cell receptors. Once in the thymic microenvironment, immature T cells express surface CD2 and CD18 (LFA-1) molecules and bind to TE cells. Thymocyte CD2 molecules interact with TE LFA-3 molecules, and thymocyte CD18 molecules interact with TE cell intercellular adhesion molecule-1 (ICAM-1) molecules. Activated TE cells produce a number of growth and differentiation cytokines (IL-1, G-CSF, M-CSF, and GM-CSF) that are capable of mediating a variety of actions on intrathymic T cell precursors. Taken together, these data suggest pathways of early T cell maturation, and define cytokines and surface molecules of TE cells that may play important roles in the early stages of T cell precursor proliferation and differentiation.

Clinical Studies-II

H 012 CLINICAL USE OF G-CSF IN JAPAN, Fumimaro Takaku, Department of Medicine, Faculty of Medicine, University of Tokyo, Hongo, Tokyo, Japan.

Human recombinant G-CSF had been used to the patients receiving chemotherapy for malignant lymphoma, lung cancer and other solid tumors. Crossover study clearly demonstrated an accelerated recovery from neutropenia after chemotherapy by G-CSF. G-CSF was also effective in accelerating the recovery from neutropenia after allogenic as well as autologous bone marrow transplantation. Administration of G-CSF to the patients with myelodysplastic syndrome and aplastic anemia induced an increase in granulocyte counts which last during the administration of G-CSF. G-CSF was also effective in treating the oppotunistic infections occurring in coupromized patients such as AIDS. Trial to treat the refractory acute leukemia cases by the combination of G-CSF and anti-leukemic chemotherapeutic agents showed that, in some cases, this combination was very effective.

Growth Factors and Megakaryopoiesis

H 100 THE MO-7 CELL LINE: A NEW MODEL TO STUDY IL-3 AND GM-CSF RECEPTORS IN HUMAN HEMOPOIETIC PROGENITORS. Gian Carlo Avanzi, Patrizia Lista, Maria F. Brizzi, Pierluigi Porcu, Arturo Rosso, Steven C. Clark and Luigi Pegoraro, Istituto di Medicina Interna dell'Universitá di Torino, Italy and Genetics Institute, Cambridge, Mass. USA. We have recently established a human leukemic cell line (M-07) with megakaryoblastic features, that shows selective growth responsiveness to IL-3 and to GM-CSF. The phenotypic characterization shows antigenic determinants common to multilineage (CD13, CD33, CD34) and to bipotent erythro-megakaryoblastic (CD36, H25) hemopoietic precursors as well as the presence of platelet glycoproteins on cell surface (CD41w and CD42w). When transferred to a serum free medium M-07 stop proliferating. The proliferative activity can be temporarily restored with the addition of h IL-3 or h GM-CSF. The responsiveness of M-07 cell line to these growth factors is dose-related and it is completely abolished by the addition of antibodies anti-IL-3 and anti-GM-CSF. Moreover, the maintenance of M-07 cell line for six months in the presence of hrIL-3, under standard culture conditions (with 10% of FCS), gave rise to a clone strictly dependent, for its survival, on IL-3. This IL-3 dependent clone shows a good response, in the 3H-TdR uptake assay, to GM-CSF. However GM-CSF itself is not able to support the growth of the same clone for more than few weeks. Our results suggest that IL-3 and GM-CSF bind to different receptors or, alternatively, that they act via independent activation mechanisms.

H 101 CHARACTERIZATION OF HUMAN GM-CSF RECEPTORS ON MALIGNANT NEURAL TISSUE, Gayle C. Baldwin, James S. Economou, Adi Gazdar, David W. Golde, and Judith C. Gasson, Division of Hematology-Oncology, Department of Medicine; Division of Surgical Oncology, Department of Surgery, UCLA School of Medicine, Los Angeles, CA 90024-1678 and Medical Oncology Branch, National Cancer Institute and National Naval Medical Center, Bethesda, MD 20014.
Human GM-CSF (granulocyte-macrophage colony-stimulating factor) stimulates proliferation of myeloid progenitors, and enhances the differentiated functions of mature hematopoietic effector cells. We recently reported GM-CSF responsiveness and receptor expression on small cell carcinoma of the lung cell lines (SCCL), which have properties of amine precursor uptake and decarboxylation (APUD) cells, and are reported to be of neuroendocrine origin. We have looked for possible GM-CSF receptor expression on a large number of other non-hematopoietic human tumor cell lines, and found binding of ^{125}I-GM-CSF to neuroendocrine tumor and melanoma cell lines. Given the neural crest origin of melanoma cells, our data indicate the presence of hematopoeitic growth factor receptors on non-hematopoietic neural tumor cell lines. Fresh cells from melanoma and paraganglioma tumors also demonstrate specific binding of ^{125}I-GM-CSF, suggesting the expression of GM-CSF receptors on non-hematopoietic cells is not a function of or restricted to long-term cell line culture. Expression of GM-CSF receptors on non-hematopoietic tumors is of clinical import, as GM-CSF is currently being used to ameliorate the myelosuppressive effects of conventional chemotherapy in patients with solid tumors.

H 102 INCREASED SURVIVAL OF HUMAN PERIPHERAL BLOOD HEMATOPOIETIC PROGENITORS CONTAINING RETROVIRAL-TRANSFERRED GENES FOLLOWING PREINCUBATION WITH HEMATOPOIETIC HORMONES, Eliel Bayever and Kathleen Haines, Allergy-Immunology-Bone Marrow Transplantation, The Children's Hospital of Philadelphia, PA 19146.
Human peripheral blood contains early hematopoietic progenitors which could be used as targets for retroviral gene transfer. This in vitro model is more easily accessible, and provides a more purified population of hematopoietic cells, the growth of which is not confounded by the effects of stromal cells which constitute the marrow microenvironment. Mononuclear cells from peripheral blood were preincubated with hematopoietic hormones which were either recombinant (erythropoietin), or conditioned media from cell-lines (Burst Promoting Activity from MOT, Hemopoietin 1 and GM-CSF from CA5637) for increasing periods of time. The cells were then infected with the retrovirus designated N2, which contains the neomycin resistance gene, and assayed in semi-solid medium for BFU-E in the presence and absence of toxic doses of G418, a neomycin analogue. Results show that preincubation of cells with CA5637-conditioned medium for at least 24 hours before exposure to virus leads to more than double the number of infected BFU-E surviving in G418. Also, there is almost double both infected and uninfected BFU-E when cultured in the absence of G418 after preincubation with CA5637-conditioned medium. Preincubation with BPA and erythropoietin also increases the number of BFU-E's, but not as much as the CA5637-conditioned medium. These results indicate that exposure of early hematopoietic progenitors from peripheral blood to hematopoietic hormones increases the integration of retroviral transferred genes as evidenced by cell survival in the presence of otherwise toxic doses of G418; the improved retroviral transfer needs to be confirmed by DNA analysis. Other manipulations of the mononuclear cell fraction of peripheral blood, such as in vitro exposure to chemotherapeutic drugs, may further improve gene transfer. Peripheral blood is a convenient source of early hematopoietic progenitors, and may be a simple model for hematopoiesis in the more complicated bone marrow.

H 103 ORDERED LOSS OF GROWTH FACTOR RESPONSIVENESS DURING THE ESTABLISHMENT OF A HUMAN IL-6 DEPENDENT CELL LINE (M-287). Maria F. Brizzi, Gian Carlo Avanzi, Patrizia Lista, Bruna Giovinazzo, Pierluigi Porcu, Steven C. Clark and Luigi Pegoraro, Istituto Di Medicina Interna dell'Universitá di Torino, Italy and Genetics Institute, Cambridge, Mass. USA. From a Ph-1 positive CML in blast crisis we derived a propagated culture of myeloid blasts which retain the ability to form colonies of partially differentiated cells in response to colony stimulating factors. The cells are Naphthyl-acetate and Naphthyl-butirate positive, they express the HLA-DR as well as antigens recognized by CD33, CD34, CD28, CD16 MoAbs, while differentiation markers such as CD11b, CD14 and CD15 are only weakly expressed. The cells of the propagated culture, in IMDM with 10% of FCS, progressively lost their proliferative activity. In serum-free conditions, the proliferative activity dropped and the cells rapidly deteriorated. However, the addition of IL-6, IL-3 or GM-CSF to serum-free cell cultures, was able to restore optimal growth conditions for different periods depending on the factor employed. The cells continuously grown in the presence of GM-CSF progressively ceased to proliferate within two months. While the cells lost their responsiveness to GM-CSF, they were still able to respond to both IL-3 and IL-6. The cells grown in the presence of IL-3, first lost their responsiveness to GM-CSF and two months later to IL-3 itself while they still retained the responsiveness to IL-6. By contrast, the cells cultured in the presence of IL-6 never lost the proliferative activity and still maintain, as the fresh cells, the ability to respond to IL-3 and GM-CSF. We conclude that M-287 cell line can provide a model to study the regulation of the hemopoietic growth factor receptors.

H 104 INHIBITORY EFFECT OF TUMOR GROWTH FACTOR-BETA (TGF-β) ON THE PROLIFERATION OF PRIMITIVE NEOPLASTIC HEMOPOIETIC PROGENITOR CELLS, J.D. Cashman, C.J. Eaves and A.C. Eaves, Terry Fox Laboratory, B.C. Cancer Research Centre, Vancouver, B.C. V5Z 1L3

In Ph^1-positive CML all compartments of hemopoietic progenitors in the blood and marrow are in a state of rapid turnover whereas in normal individuals the cells in many of these compartments are quiescent. Mechanisms that permit CML progenitors to escape regulatory influences governing the proliferative state of normal progenitors are not understood. Recently, we showed that primitive normal progenitors repeatedly return to a quiescent state in vitro when co-cultured with normal marrow mesenchymal cells. Under the same conditions, analogous populations of CML cells continue to proliferate. Further studies showed that the addition of various mesenchymal cell activators to established normal long-term marrow cultures stimulates the majority of the quiescent primitive hemopoietic cells present to re-enter S-phase. However, this stimulation, which appears to be indirect, can be overcome by the simultaneous addition of 5 ng/ml of TGF-β, an agent that has a selective, but direct inhibitory action on primitive normal progenitors. To determine whether TGF-β can also inhibit the proliferation of CML cells, we first examined the effect of TGF-β on colony growth in methylcellulose. From 0.2-15 ng/ml of TGF-β inhibited all classes of CML progenitors, but high proliferative potential progenitors were affected most severely. Preliminary experiments indicate that TGF-β can also reversibly arrest the cycling of primitive CML progenitors in long-term blood cultures set up with or without a normal marrow feeder layer. These results suggest that CML progenitors are normally responsive to the direct, inhibitory effects of TGF-β and that their failure to become quiescent in the presence of mesenchymal cells must be explained by a defect in another regulatory mechanism.

H 105 GM-CSF INDIRECTLY DOWN-REGULATES HIGH-AFFINITY LTB_4 RECEPTOR EXPRESSION BY DIRECTLY STIMULATING NEUTROPHIL LTB_4 SYNTHESIS, John F. DiPersio, Cyrus Hedvat, and Judith C. Gasson, Divison of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024-1678

Previous studies by our laboratory have shown that GM-CSF primes neutrophils for enhanced release of arachidonic acid, leukotreine B_4 synthesis, enhanced cellular activation, Na^+/H^+ antiport activity, and calcium fluxes in response to secondary stimuli such as chemotactic factors and ionophores. A direct effect of GM-CSF has been difficult to demonstrate. GM-CSF, independent of secondary stimuli, induces a dramatic decreased expression of the high-affinity (Kd 1-2 nM) LTB_4 receptor. This effect by GM-CSF is inhibited by pre-incubating neutrophils with NDGA (1-50 μM), a potent inhibitor of the 5-lipoxygenase pathway. Consistent with this result are recent studies using a modified neutral lipid extraction followed by a highly sensitive and specific LTB_4 radioimmunoassay demonstrating that GM-CSF (≥200 nM) induces a 2-5X increase in neutrophil LTB_4 synthesis (baseline, 10-40 pg/10^7 cells; stimulated, 50-400 pg/10^7 cells). The conditions determined for maximal neutrophil LTB_4 synthesis by GM-CSF correlate with GM-CSF's effect on down-regulating the high-affinity LTB_4 receptor.

These studies suggest that GM-CSF may prime neutrophils by directly inducing the increased synthesis and release of LTB_4 in sufficient levels to occupy and down-regulate the high-affinity LTB_4 receptor. The mechanisms by which GM-CSF induces increased synthesis of LTB_4 are currently under investigation.

H 107 A MODEL FOR THE STUDY OF LINEAGE DETERMINATION IN HL-60 HUMAN LEUKEMIA CELLS, Steven A. Fischkoff and Randall M. Rossi, Hematology/Oncology Section, Philadelphia VA Medical Center and University of Pennsylvania School of Medicine, Philadephia, PA 19014

In order to develop a model system with which to study the early events leading to lineage commitment in myeloid precursors, we have characterized various cloned sublines of HL-60 human leukemia cells. Of these lines, several mature almost exclusively to either neutrophils, monocyte/macrophages or eosinophils when induced to mature with butyric acid (or allowed to spontaneously mature) under identical culture conditions. In addition, a number of clones were found to mature to mixtures of two, or all three, of the possible lineages. Despite the lineage "direction" of these clones, each was inducible to mature to neutrophils with DMSO and to monocyte/macrophages with 1,25-$(OH)_2$ Vitamin D_3. However, the ability of DMSO or 1,25-$(OH)_2$ Vitamin D_3 to "switch" the lineage decision diminshed with time during butyrate induced maturation. Looking with multiple markers, no mature cells with characteristics of more than one lineage were found. We conclude that lineage commitment is a multi-step process in HL-60 cells with stable, identifiable, intermediate stages. The early steps appear to occur through a probablistic mechanism. Irreversible lineage commitment appears to be temporally related to maturation commitment. In mature HL-60 cells, lineages are purely expressed, though proteins characteristic of more than one lineage may be expressed in immature cells.

H 108 INDUCTION OF IMMUNOREGULATORY MACROPHAGES BY A COLONY STIMULATING LIKE FACTOR PRODUCED BY MAMMARY TUMORS. Yangxin Fu, Yang Wang, Joaquin Jimenez, Laphalle Fuller and Diana M. Lopez, Department of Microbiology and Immunology and Medicine, University of Miami School of Medicine, Miami, FL 33101

Splenic macrophages of a MAC 1^+ 2^+ phenotype from mammary tumor bearers are capable of down regulating lymphocyte responses to mitogens and tumor associated antigens by alterations of antigen presenting functions. The kinetics of accumulation of these cells in spleens parallels the growth of the tumor and the concomitant immunosuppression. A dramatic increase of macrophage progenitors could be detected in the tumor bearer spleens. Likewise, a rise of granulocytes and macrophages could be detected in the blood and bone marrow. Furthermore, an increase of a GM-colony stimulating like activity (CSA) was observed in the sera of tumor bearers. Even higher levels of the CSA were found in tumor cystic fluid, suggesting that this factor may be derived from the tumor. Supernatants from in vitro cultures of the tumor cells grown in serum free media displayed strong CSA. Partial purification of this material by concentration in Centri Cell, and fractionation in Sephacryl 300 and FPLC indicated that the m.w. of the active fraction is 32-38,000. Colonies obtained by incubation of this factor with bone marrow cells have a morphology similar to GM-CSF. The macrophages from bone marrow cells cultured 3 days in vitro with the tumor cell superntants strongly inhibit normal splenocytes proliferative responses to mitogens. This inhibitory effect could not be reversed by indomethacin. In vivo studies explored the effect of daily injection of tumor cystic fluid into normal mice. Such a treatment mimics the hemopoietic and immunologic alterations observed in tumor bearers. We hypothesize that the tumor releases factors that are capable of expanding MAC 1^+2^+ immunoregulatory cells which contribute to the immune suppression in tumor bearing mice.

H 109 EFFECT OF NEUROTOXIN 6-HYDROXYDOPAMINE (6-OHDA) ON MEGAKARYOCYTE PROGENITORS (CFU-MEG), B.G. Gordon and J.G. Sharp, Departments of Pediatrics and Anatomy, University of Nebraska Medical Center, Omaha, NE 68105. Catecholaminergic neurons and neuroblastoma (NBL) cells possess a specific membrane transport system for the re-uptake of catecholamines. 6-OHDA, an autooxidizing toxin is concentrated in catecholaminergic cells by this transport system and preferentially kills such cells. 6-OHDA has been used to purge NBL cells from human bone marrow, in preparation for autologous marrow transplantation. Previous studies, assessing viability by trypan blue exclusion, have demonstrated no toxicity of this agent to bone marrow mononuclear cells. However, effects on specific subsets of marrow cells were not examined. Platelets, megakaryocytes and possibly their less mature precursors are also capable of active uptake of catecholamines, therefore this agent may also be toxic to these cells. We have examined the effects of 6-OHDA on CFU-Meg assayed in semi-solid agar. BDF1 mouse marrow cells were treated with varying concentrations of 6-OHDA. The CFU-Meg assay was performed using WEHI and MC-1 conditioned medium (containing at least IL-3, GM-CSF, G-CSF and M-CSF) as stimulatory activity. Groups of three or more acetylcholinesterase positive cells were counted as megakaryocyte colonies. In parallel experiments, cells from the human NBL line SK-N-MC were also treated with 6-OHDA, and viability assessed by trypan blue exclusion. We found a dose dependent decrease in the number of CFU-Meg, with 90% inhibition between 10 and 20 ug/ml 6-OHDA, which was the same as the LD90 for NBL cells. This suggests that CFU-Meg are as sensitive to 6-OHDA as NBL cells and patients transplanted with marrow treated with 6-OHDA may show delayed megakaryocyte engraftment and platelet reconstitution. (Supported by a grant from the Edna Ittner Pediatric Research Support Fund.)

H 110 SYNTHESIS OF GRANULOCYTE COLONY-STIMULATING FACTOR AND ITS REQUIREMENT FOR TERMINAL DIVISIONS IN CHRONIC MYELOGENOUS LEUKAEMIA, Friedhelm Herrmann, Helga Klein, Margitt Wieser, Albrecht Lindemann, Wolfgang Oster, Roland Mertelsmann, Engelhard Schleiermacher+, Reinhard Becher* and Larry Souza#, Department of Haematology, University of Mainz, D-6500 Mainz, FRG, + Institute for Anthropology, University of Mainz, D-6500 Mainz, FRG, * Department of Medicine, Tumour Research, University of Essen, D-4300 Essen, FRG, # Amgen, Thousand Oaks, CA 91320/805

Chronic myelogenous leukaemia (CML) is a clonal disorder believed to originate in a primitive haematopoietic stem cell. Since the proliferative potential of myeloid progenitor cells (MPC) from stable phase CML patients is considerably impaired compared to the proliferative capacity of MPC derived from normal human bone marrow, it seems to be likely, that in CML malignant cells are expanded in later maturational compartments (i.e. promyelocytes/monocytes). Recently, it has been shown that production of growth factors occurs in some malignant populations of both myeloid and lymphoid neoplasms leading to autonomous growth. Using mRNA hybridization and biologic assays we show that maturing peripheral blood and bone marrow cells from CML patients constitutively produce granulocyte colony-stimulating factor (G-CSF), a molecule known to stimulate growth and differentiation of normal granulocyte progenitor cells. Furthermore, we report that CML cells expressing G-CSF use this factor to stimulate terminal divisions in maturing leukaemic cells. In blastic phase, CML cells failed, however, to express the G-CSF gene unless pertubation of the G-CSF gene had resulted in its constitutive expression, as demonstrated in samples of blastic phase CML which exhibited the isochromosome 17q.

H 111 **HUMAN PLATELETS EXPRESS HIGH LEVELS OF $pp60^{c\text{-}src}$ and $p60^{fyn}$**, I.D. Horak[1,2], M.L. Corcoran[3], P.A. Thompson[1,4], L.M. Wahl[3], and J.B. Bolen[1], [1]Laboratory of Tumor Virus Biology, [2]Medicine Branch, [4]NCI-Navy, National Cancer Institute, and [3]Laboratory of Immunology, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892. Human platelets possess high levels of tyrosine-specific protein kinase activity which has been attributed largely to the expression of $pp60^{c\text{-}src}$. We have found that in addition to $pp60^{c\text{-}src}$, one other member of the src family of tyrosine protein kinases, $p60^{fyn}$, is also expressed at high levels in plateletes. The abundance of $pp60^{c\text{-}src}$ in platelets was estimated to be approximately 4- to 5-fold higher than the level of $p60^{fyn}$. Thrombin-mediated activation of platelets was found to stimulate the activity of both $pp60^{c\text{-}src}$ and $p60^{fyn}$ 2- to 4-fold. These observations imply that at least two members of the src gene family, $pp60^{c\text{-}src}$ and $p60^{fyn}$, participate in human platelet signal transduction events.

H 112 **IL-6 INTERACTS WITH INDIVIDUAL HEMOPOIETIC GROWTH FACTORS TO SELECTIVELY ENHANCE THE GROWTH OF DISTINCT HEMOPOIETIC PROGENITOR CELLS**, Susan Hudak, John Jackson, Donna Rennick, Department of Immunology, DNAX Research Institute of Cellular and Molecular Biology, Palo Alto, CA 94304. The growth promoting activities of mouse IL-6 in combination with either M-CSF, G-CSF, GM-CSF, IL-4, or IL-3 were assessed in semi-solid mouse bone marrow (BM) cultures. To determine the effects of different factor combinations on the growth of hemopoietic cells at different stages of maturation, BM was obtained from normal and 5-fluorouracil (5-FU)-treated mice. We found that only IL-6 and IL-3 synergistically enhanced colony formation by multipotential cells in day-2 and day-4 post-5-FU BM cultures. These results are consistent with other studies using human IL-6 (Ikebuchi, J. et al). When day-4 post-5-FU BM cells were used, all factors interacted with IL-6 to stimulate increased numbers of giant granulocyte/macrophage colonies. Many of these colonies attained a diameter of ≥0.5 mm suggesting that they derive from high proliferative potential cells (HPP-CFC). When normal or day-8 post-5-FU BM cells were used, the combination of IL-6, IL-4, and Epo stimulated twice as many erythrocyte colonies as IL-4 and Epo. IL-6 also enhanced IL-3-dependent megakaryocyte colony formation. Pure erythrocyte and megakaryocyte colonies were absent from cultures of day-2 and day-4 post-5-FU BM stimulated with the same factors. These results indicate that IL-6 interacts with other factors besides IL-3 to enhance the growth of hemopoietic progenitors. Further, different types of progenitor cells are responsive to the actions of different factor combinations.

H 113 IL-3-MEDIATED IL-6 PRODUCTION IN MURINE IL-3-DEPENDENT HEMOPOIETIC CELL LINES. L.Hültner, H. Szöts, M.Welle, J.Moeller, P.Dörmer. GSF-Institut für Experimentelle Hämatologie, Munich, FRG.

A series of murine IL-3-dependent hemopoietic cell lines were studied for their capacity to produce IL-6 in vitro. These included a bone marrow-derived mast cell line (L138.8A) and several myeloid cell lines described in the literature (DA-1, DA-3, NFS-60, NFS-78, FDC-P1, 32Dcl.23). All of these cell lines produced growth factor activity for IL-6-dependent hybridoma cells (7TD1), which was completely neutralized by the monoclonal anti-IL-6-antibody 6B4. In each case IL-6 gene expression was also evident at the mRNA level using a murine IL-6-specific cDNA probe (clone pHP1B5; van Snick et al. 1988). In 32Dcl.23 cells (2 x 10^5/ml) stimulated for 24 hours with serial dilutions of purified murine IL-3 a positive correlation was found between the IL-3 dose and the amount of IL-6 measured in the conditioned media. At the same time (24h) this correlation was not evident at the mRNA level. However, prolonged exposure of 32Dcl.23 cells (up to 72 hours) to either a high (60 U/ml) or a low concentration of IL-3 (1 U/ml) revealed a time-dependent increase and decrease, respectively, of IL-6 mRNA levels. At both IL-3 concentrations 32Dcl.23 cells remained in a fully viable and proliferative state. The influence of IL-3 on IL-6 release could be specifically counteracted by anti-IL-3-antiserum.

H 114 **HEMATOPOIETIC GROWTH FACTOR-STIMULATED PHOSPHORYLATION OF A 92KD CELLULAR PROTEIN IN ACUTE MYELOGENOUS LEUKEMIA CELLS, Richard D. Huhn, Departments of Hematology/Oncology and Biochemistry, St. Jude Children's Research Hospital, Memphis, TN 38101.**

Hematopoietic growth factors support the proliferation and differentiation of specific lineages of blood cells *in vitro* and *in vivo*. Interleukin-3 (IL-3) supports multi-lineage hematopoiesis, while GM-CSF supports primarily myeloid lineages. The receptor for murine IL-3 is thought to be a protein-tyrosine kinase,[1] but little is known of the signal transduction pathways for either human IL-3 or GM-CSF. The AML-193 myeloid leukemia cell line[2] responds with enhanced proliferation to either rhIL-3 or rhGM-CSF. Stimulation of AML-193 cells with either rhGM-CSF or rhIL-3 after metabolic labeling with ^{32}P-phosphate results in the specific phosphorylation of a 92kd protein isolated by affinity chromatography on 1G2 antiphosphotyrosine antibody.[3] This response is observed at concentrations of less than 10U/ml for both rhIL-3 and rhGM-CSF. The relationship of this protein to other protein-tyrosine kinase oncogene proteins and substrates, its phosphorylation kinetics and specificity of growth factor response, and its role in growth factor signal transduction are under investigation.

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[1]Isfort R., *et al*, J. Biol. Chem. (In press), 1988.
[2]Santoli, D., *et al*, J. Immunol. 139:3348, 1987.
[3]Huhn,R., *et al*, Proc. Natl. Acad. Sci. USA 84:4408, 1987.

H 115 ENDOGENOUS AND EXOGENOUS CSF-1 STUDIED IN HUMAN LONG-TERM BONE MARROW CULTURES (LTMC) A. Janowska-Wieczorek, J. Tupas, I. Friesen, I. Jass, H. Mayani, A.R. Turner, L.J. Guilbert, Department of Medicine, University of Alberta and Canadian Red Cross, Blood Center, Edmonton, Canada.

Even while clinical trials are under way, the role of myeloid-lineage specific factors such as CSF-1 in the maintenance of normal, human long-term hematopoiesis in culture remains unclear. Neither the kinetics of endogenous production nor the effects of added factor have been established in human LTMC. We therefore examined the role of CSF-1 in LTMC maintained for up to 48 weeks. Non-adherent cells were evaluated weekly for differential morphology and for number of myeloid progenitors (CFU-GM). Levels of CSF-1 in the cultures were measured by specific radioreceptor assay (RRA). During the first few months of culture, CSF-1 levels were low and physiologic (2-5 ng/ml). Thereafter, levels underwent reproducible oscillations, reaching peaks of >7 ng/ml. The first oscillation occurred about 3 weeks before numbers of CFU-GM began to decline, and another occurred after active myelopoiesis ceased. Addition of pure recombinant CSF-1 (from S. Clark, G.I.) at 10-25 ng/ml, daily for a week, to LTMC maintained CSF-1 levels at between 15 to >30 ng/ml for that week only and triggered decline of CFU-GM within the next month. The kinetics of the decline were a function of the amount of CSF-1 added. These data indicate that during *in vitro* active hematopoiesis in LTMC, CSF-1 is maintained at levels similar to those found *in vivo* and that higher levels predict decline of active hematopoiesis. The observation that decline could be induced earlier with exogenous CSF-1 suggests a causative role and provides evidence for an important function for CSF-1 in hematopoietic homeostasis.

H 116 **IL-3 AND STROMAL CELL DERIVED FACTOR SYNERGISTICALLY STIMULATE THE GROWTH OF A PRE-B CELL LINE CLONED FROM A LONG-TERM LYMPHOID BONE MARROW CULTURE,** John D. Jackson, Donna M. Rennick, Courtney Moulds, Frank Lee and Gloria Yang, Departments of Immunology and Molecular Biology, DNAX Research Institute of Cellular and Molecular Biology, Palo Alto, CA 94304. The addition of IL-3 to modified Whitlock-Witte long-term lymphocyte cultures (LTLCs) enhanced the growth of a small but significant number of B cell precursors which were supported by an adherent stromal cell layer. Several pre-B cell lines were cloned from the IL-3-treated LTLCs. One cloned line, BL-3, exhibited a sIg^-, $B220^+$ phenotype and μ heavy chain rearrangement. BL-3 cells were unresponsive to IL-3 except when it was used at high concentrations. In contrast, significant growth was stimulated by stromal cell conditioned medium (CM), however, optimal growth of BL-3 was stimulated by stromal cell CM plus IL-3. Synergy between the stromal cell-derived factor and IL-3 occurred when IL-3 was used over a wide range of concentrations including a low concentration which was ineffective as a growth stimulus alone. The finding that more than one factor is required to sustain optimal growth of some pre-B cells parallels the complex growth requirements reported for some primitive myeloid/erythroid progenitors.

H 117 PRIMARY RESPONSES OF PURIFIED HUMAN HEMOPOIETIC PROGENITOR CELLS TO RECOMBINANT GROWTH FACTORS. Steven W. Kessler, Steven C. Clark, and Carl H. June. Naval Medical Research Institute, Bethesda, MD, and Genetics Institute, Cambridge, MA.
Hemopoietic recombinant growth factors (rGFs) may induce proliferation or differentiation of progenitor cells by direct or indirect mechanisms, but direct effects have been difficult to show. We studied early metabolic and mitogenic responses to rGFs of human CD34+ progenitors purified to a level (>99.95%) which minimized indirect effects of contaminating cells. Cell recovery was achieved by large-scale positive immunomagnetic selection from cadaveric bone marrow under conditions which preserved surface membrane integrity. We measured intracellular calcium levels in single cells loaded with Indo-1 and analyzed by flow cytometry. Typical responses were seen with ionomycin, but no changes were seen during 60 min exposures to IL-1a, IL-3, IL-6, GM-CSF, G-CSF, M-CSF, or Epo, or to a variety of lectins which bound to these cells. These findings suggest CD34+ progenitors are too immature to use this signalling mechanism with known rGFs. We also studied kinetics of induction of progenitors into cell cycle ($S+G_2/M$) by flow cytometric analysis of DNA. Under serum-free conditions IL-3 and to a minor degree Epo were stimulatory by 24 hr, and by 96 hr these rGFs along with G-CSF and M-CSF (but not GM-CSF, IL-1a, or IL-6) were inductive. IL-1a, IL-6, GM-CSF, G-CSF, and Epo were additive or synergistic with IL-3 (>40% of cells in cycle by 96 hr), as was GM-CSF with Epo. Direct GM-CSF activity required an unidentified serum component. In addition, we studied mitogenicity in serum-free medium by kinetics of ^{3}H-thymidine incorporation. Only cultures containing IL-3 had marked early and persistent mitogenic activity; effects of several other rGFs, including GM-CSF, were minor and not apparent until days 4-5. These studies identify IL-3 as the primary mitogen for most CD34+ progenitors, with the other rGFs acting as co-stimulators by undefined mechanisms.

H 118 CHARACTERIZATION OF A NOVEL MULTI-FACTOR DEPENDENT HUMAN CELL LINE, TF-1, AND THE ANALYSIS OF THE RECEPTORS FOR EPO, GM-CSF AND IL-3, Toshio KItamura, Shigeru Chiba, Tomoaki Kuwaki, Chie Misawa, Arinobu Tojo, Kohei Miyazono, Akio Urabe and Fumimaro Takaku, The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo, Japan 113.
We established a novel multi-factor dependent human cell line, TF-1, from a patient with erythroleukemia. TF-1 cell line showed strict growth dependencies on Epo, GM-CSF and IL-3. IL-5 also sustained the proliferation of this cell line, and IL-4 and IL-6 extended their survival. TGF-β and interferon-α ,β and γ inhibited the factor-dependent growth of TF-1, except that interferon-γ did not inhibit the IL-6-dependent growth. On the other hand, IL-1 greatly enhanced the factor-dependent growth of TF-1. Altough these hemopoietic growth factors and interleukins could not induce any apparant differentiation, TPA induced a drastic differentiation into macrophages, hemin and δ -ALA induced the synthesis of hemoglobin. Next, we investigated the receptors for Epo, GM-CSF and IL-3 by Scatchard analysis and chemical cross-linking studies. The modulation of these receptors by TPA, δ -ALA and IL-1 will be discussed.

Abstract Withdrawn

H 120 G-CSF INDUCED COMPETENCE AND MEMORY TO RESPOND TO GM-CSF IN AN IL3-DEPENDENT MURINE HEMOPOIETIC PRECURSOR CELL LINE, Brent L. Kreider, Michael B. Prystowsky*, Paul D. Phillips, and Giovanni Rovera, The Wistar Instituite of Anatomy and Biology, Philadelphia, PA 19104, and * University of Pennsylvania, Philadelphia, PA 19104. 32DC13(G) is an IL3-dependent murine cell line which readily differentiates into neutrophilic granulocytes upon exposure to granulocyte colony stimulating factor (G-CSF). 32DC13(G) show no immediate proliferative response to recombinant murine granulocytic-macrophage colony stimulating factor (rmGM-CSF), although rare clones able to grow in rmGM-CSF can be obtained upon prolonged exposure. A very different rmGM-CSF response is seen after exposure to G-CSF. When 32DC13(G) cells are treated with G-CSF for a minimum of three days, removed from the G-CSF containing medium and exposed to rmGM-CSF, the cells are now capable of proliferating in response to the rmGM-CSF. There is a rapid expansion of the cell pool with representatives of both the neutrophilic granulocyte and monocytic lineages present. Concomitant with the ability of 32DC13(G) cells to respond to rmGM-CSF is the appearance of high affinity GM-CSF receptors on their cell surface. 32DC13(G) cells primed with G-CSF not only have the immediate capability to proliferate in rmGM-CSF but also remain competent to respond to rmGM-CSF for a very extended period of time even if the cells are maintained in mIL-3 containing medium after G-CSF exposure. In addition, cells growing in rmGM-CSF will complete their differentiation along the neutrophilic granulocyte lineage when returned to G-CSF containing medium. Thus, G-CSF induction of 32DC13(G) cells results in their ability to respond to rmGM-CSF, conceivably due to the appearance of the GM-CSF receptor on their cell surface, and this competence is maintained even after long term growth in mIL-3.

H 121 **IN VITRO ACTIONS OF RECOMBINANT LYMPHOKINES ON HAEMOPOEITIC PROGENITOR CELLS.** **Y.L.Kwong, J.L.Millar, R.L.Powles. Institute of Cancer Research,Royal Marsden Hospital, SM2 5NG, U.K.**

The effects of rhIL3(Glaxo),rhGM-CSF(Sandoz),rhG-CSF(Glaxo) & rhILlα(Roche) on the in vitro survival & proliferation of haemopoietic progenitor cells were studied. Non-adherent, T cell depleted normal human marrow mononuclear cells were incubated in suspension culture with these lymphokines, either alone or in combinations. At various time points (up to 1 week), cells were harvested and number of clonogenic progenitor cells determined by agar culture. Against a steady decline of clonogenic cells in controls, incubations with lymphokines resulted in increases of CFU-C (with preservation of colony size) which peaked at 3-4 days, respective increases being 2.0 with G-CSF,3.2±1.4 with ILlα, 3.6±1.2 with GM-CSF & 7.4±2.6 with IL3 (n=6). Combinations of lymphokines did not show significant differences. Decline of clonogenic cells occurred in all groups after 4 days. To examine whether this expansion of clonogenic cells represents genuine primitive progenitor cell renewal or simply differentiation from a more primitive pool into the CFC compartment,a similar murine system using recombinant murine lymphokines (except h G-CSF) was investigated.In GM-CSF,G-CSF & ILlα groups, while CFU-C followed a similar pattern, CFU-S declined,albeit at a slower rate than controls.When IL3 was used in the suspension culture,an increase in CFU-S resulted, reaching a peak at Day 3 (mean 1.4±0.2). Addition of GM-CSF,ILlα & G-CSF to IL3 resulted in further increases in CFU-S, although the differences were not statistically significant. These results indicate that while haemopoietic growth factors are needed for in vitro survival and proliferation of clonogenic progenitor cells, IL3 is needed for self renewal of primitive progenitor cells, at least at the level of a multipotential stem cell.

H 122 EPITOPE MAPPING OF HUMAN GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR,

Judith E. Layton, Peter J. Dempsey, Edouard C. Nice, Louis J. Fabri, Cui Da-fu, Richard J. Simpson, George Morstyn and Antony W. Burgess,
Ludwig Institute for Cancer Research, Melbourne, Australia 3050.

One approach to identifying functionally active regions of the GM-CSF molecule is to map the epitopes recognised by neutralizing anti-rhGM-CSF antibodies. We have defined the epitope recognised by one such monoclonal antibody (LMM102) using proteolytic fragments obtained by enzymatic digestion of rhGM-CSF (Schering Plough). RP-HPLC fractionation of the tryptic digest resulted in the identification of an antibody-binding "tryptic core" containing 66 amino acids (52% of the molecule) including both disulphide linkages. Further digestion with S. aureus V8 protease produced only one immunoreactive product that was shown to consist of two peptides from the C-terminal region, joined by the disulphide bond between residues 88 and 121. Following reduction with DTT, the two individual peptides which were purified, were no longer reactive with the antibody. An analogue of this peptide (26 amino acids) has been synthesized and found to have the same immunoreactivity as the enzymic digestion product. Modified peptides have been chemically synthesized to further localize the epitope. These are being tested for biological activity using a mitogenic assay (using the GM-CSF-dependent AML193 cell line) and a receptor competition assay.

H 123 PROTO-ONCOGENE EXPRESSION AND DISSECTION OF THE MYELOID GROWTH TO DIFFERENTIATION SIGNAL TRANSDUCTION CASCADE. D. Liebermann and B. Hoffman-Liebermann, Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Phil, PA 19104. Using physiological inducers of myeloid cell growth and differentiation, the expression of the proto-oncogenes c-myc, c-myb, c-fos, c-fes and c-fms was analyzed during normal myelopoiesis, where growth is coupled to differentiation and in leukemia, where growth has been uncoupled from differentiation, as well as upon suppression of the leukemic phenotype. Proto-oncogene expression also was used as a tool to dissect the growth to differentiation developmental cascade. Myeloid cell growth was correlated with high c-myc and c-myb RNA levels, which were down-regulated and eventually reduced to undetectable levels following induction of differentiation. No c-myc RNA is detected when normal myeloid progenitors were induced for differentiation in the absence of growth, suggesting that c-myc may play no or an inhibitory role in differentiation. RNA levels of c-fos, c-fes and c-fms were undetectable in normal or M1D+ leukemic myeloblasts, and stably induced upon stimulation of the normal precursors for growth and differentiation. Only c-fes RNA was induced upon M1D+ differentiation. Using c-myc and c-myb RNA suppression as molecular markers for induction of M1D+ differentiation, the existence of myeloid differentiation factor(s) distinct from growth factors has been demonstrated. Such differentiation inducing activity was found in media conditioned by mouse lungs or granulocytes, and induced in normal myeloid precursors by the myelopoietic growth factors IL3, GM-CSF, G-CSF, and M-CSF. These studies indicate that during myelopoiesis myeloid growth factors induce myeloid differentiation factors, subsequently suppressing c-myc and c-myb RNA expression, leading to the induction of differentiation and growth arrest. This cascade is impaired in leukemia. Studies are currently underway to determine the role of the various proto-oncogenes by perturbing their expression and then monitoring any effects on growth and differentiation of normal and leukemic myeloid cells.

H 124 EFFECTS OF IL-3 AND ARA-C COMBINED TREATMENT ON AML BLASTS AND NORMAL MYELOID PROGENITORS. Patrizia Lista, Maria F. Brizzi, Gian Carlo Avanzi, Pierluigi Porcu, Mario Rossi, Steven C. Clark and Luigi Pegoraro. Istituto di Medicina Interna dell'Universitá and Ospedale Maggiore S. Giovanni, Torino, Italy - Genetics Institute, Cambridge, Mass., USA. The proliferative compartment of acute leukemia blast populations is refed by more immature quiescent "stem" cells which, because of its resistance to radiations and cytotoxic agents are considered responsable of clinical relapses after therapy. We have recently shown that hemopoietic growth factors can effectively recruit quiescent AML blasts into cycle. On these bases, a combined treatment with IL-3 and ara-C was studied, with the aim of improving the in vitro cytotoxic effect of this cycle-specific drug on AML populations, by increasing their proliferative activity. Leukemic cells from AML patients were incubated for two days in liquid culture, in the presence or absence of the human recombinant IL-3 (10 U/ml), and subsequently exposed to Ara-C (3 ug/ml) for 24 hours. The number of residual leukemic stem cells was evaluated by a clonogenic assay. The results showed that, in those cases which were good responders to IL-3 in the 3H-TdR uptake assay, Ara-C exposure eliminated a greater proportion of clonogenic cells in IL-3 pre-treated samples than in the untreated ones ($p < 0.001$), while in IL-3 unresponsive cases the difference was not significant. Moreover, when this same protocol was applied to normal bone marrow cells, IL-3 pre-treatment was not found to increase the cytotoxicity of ara-C on CFU-GM. The results indicate that IL-3 may improve the terapeutic index of cycle-specific drugs in AML therapy.

H 125 EFFECTS OF EXOGENOUS ERYTHROPOIETIN ON HUMAN BONE MARROW CELLS IN LONG-TERM CULTURE. Hector Mayani & Anna Janowska-Wieczorek. Department of Medicine, University of Alberta, and Canadian Red Cross Blood Center, Edmonton, Canada.

Although long-term bone marrow cultures (LTBMC) more closely reproduce hemopoiesis than any other *in vitro* method, they are deficient in red blood cell production, lacking by week 3 of culture almost all the morphologically recognizable erythroid cells. The deficiency seems to be at the level of maturation of CFU-E from BFU-E. Since erythropoietin (Epo) is known to preferentially act on CFU-E and early erythroblasts and since Epo production has not been demonstrated in LTBMC, we asked whether addition of Epo would restore erythropoiesis in the non-adherent layer of human LTBMC. We therefore, compared LTBMC established from the same bone marrow donor,cultured for ten weeks with and without human Epo (1 u/ml; recombinant Epo, gift of S. Clark, Genetics Inst; or urinary Epo). We found that Epo had no effect on CFU-MIX kinetics, accelerated loss of BFU-E, transiently sustained CFU-E and erythroblast levels, but did not restore erythropoiesis to *in vivo* proportions.

Ratio of Erythroid Progenitors to CFU-MIX

	CFU-MIX		BFU-E		CFU-E		Erythroblasts	
Epo	-	+	-	+	-	+	-	+
Normal Marrow	1		4.7		7.3		6396	
LTBMC - 3 wks	1	1	12.2	9.2	0.1	3.4	3000	10000
LTBMC - 5 wks	1	1	9.4	3.3	0	0.6	0	1900
LTBMC - 7 wks	1	1	2.3	0	0	0	0	0

Thus, Epo promotes the maturation step of BFU-E to CFU-E in LTBMC at the expense of BFU-E levels, however, it is not able to reverse the inefficient expansion of later erythroid precursors which typify LTBMC's.

H 126 STIMULATION OF PRIMITIVE PROGENITOR CELLS BY FACTORS PRODUCED BY 5637 CELLS: COMPARISON TO INTERLEUKIN-1 ALPHA, Ian McNiece, Tony Kriegler and Peter Quesenberry, Dep. of Internal Medicine, Univ. of Virginia, Charlottesville, VA and Peter MacCallum Cancer Inst. Melbourne, Australia. A synergistic factor produced by the human bladder carcinoma cell line 5637 (SF-1) stimulates primitive bone marrow progenitor cells termed HPP-CFC, in the presence of an optimal dose of CSF-1. Recent reports have demonstrated that IL-1 alpha is identical to hemopoietin 1 and have suggested that IL-1 is the synergsitic factor present in 5637 cm. We have compared the ability of recombinant IL-1 alpha and partially purified preparations of SF-1 to synergise with optimal doses of CSF-1 to stimulate HPP-CFC. In all experiments the numbers of HPP-CFC colonies formed with IL-1 were significantly less than with SF-1. Replating experiments demonstrated that SF-1 plus CSF-1 generated HPP-CFC responsive to IL-3 plus CSF-1, however, IL-1 plus CSF-1 resulted in no generation of HPP-CFC. Multiple factor combinations of IL-1 and SF-1 with rhG-CSF, rmGM-CSF or rhGM-CSFand CSF-1 also resulted in less HPP-CFC colony formation in cultures containing IL-1 compared to SF-1. However, IL-1 synergised with rhG-CSF, rmGM-CSF and CSF-1 to stimulate approximately 12 HPP-CFC colonies per 50,000 BM cells. These data suggest the presence of a factor in 5637 cm which is distinct from G-CSF and GM-CSF which synergises with IL-1 to produce the SF-1 effect. As 5637 cells produce IL-6, antibody to IL-6 was added to SF-1 and no decrease in HPP-CFC colony formation occurred. The addition of IL-6 to combinations of G-CSF, GM-CSF, CSF-1 and IL-1 resulted in no increase in either total or HPP-CFC colonies. However, the addition of IL-6 to IL-1 plus CSF-1 plus IL-3 resulted in an increase of 300% in HPP-CFC colonies. This increase could be ablated by the addition of anti-IL-6.

H 127 SYNERGISTIC EFFECT OF IL_6 (IFN-β_2) AND IL_3 ON EARLY HEMATOPOIETIC PROGENITORS GROWTH. Rita Michalevicz, David Lifshitz and Michel Revel, Hematology Institute, Tel-Aviv Medical Center and Virology Department, Weizman Institute, Israel.

Bone marrow progenitor cells (CD_{34} positive, T and monocyte depleted) were treated with 4HC, and their growth response to IL_6, IL_3 and their combination compared to that of IL_1 and IL_3. rIL_6 (IFN β_2) alone had no effect on the growth of early non-cycling stem cells but when used together with IL_3 promoted an increase in the number of multipotential and erythroid precursors. This effect was comparable to that of IL_1 and IL_3. Two systems namely liquid and day 0 cultures were compared assuming that earlier cells are detected in the liquid culture system.Indeed, different responses were obtained: IL_6 on day 0 cultures promoted growth of GM-colonies but not multipotential ones. To check whether the IL_1, IL_3 effect on early progenitor bone marrow cells was through IL_6 we have used a monoclonal antibody to IL_6. Our results show that the effects of IL_1 are probably not due to IL_6 since anti-IL_6 did not inhibit growth response of progenitor cells in cultures with IL_1 and IL_3. These results are in accordance with a previous report and explore further the effects of IL_6 on both early and late progenitors as well as on marrow versus peripheral blood stem cells. We conclude that rIL_6 (IFN-β_2) in conjunct with IL_3 may be of value in the context of bone marrow transplantation.

H 128 IDENTIFICATION OF A SERIES OF DIFFERENTIATION-ASSOCIATED GENES FROM GM-CSF STIMULATED MURINE BONE MARROW, Lynn C. Moscinski and Michael B. Prystowsky, Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104. Partially purified granulocyte-macrophage colony-stimulating factor (GM-CSF) has been used to stimulate proliferation and differentiation of murine bone marrow progenitor cells. From data obtained by studying the time course of RNA expression of myeloperoxidase and several known proto-oncogenes (c-myb, c-myc, c-fos), the promyelocyte stage of differentiation (day 3 post GM-CSF stimulation) was identified as a pivotal point in myeloid maturation. (Oncogene 2:167, 1988) A cDNA library was constructed from murine bone marrow cells harvested at this time point, and differentially screened with cDNA prepared from both day 3 marrow and proliferating murine EL4 T cells. From approximately 80 potential early myeloid specific clones, three (C15, C9, B9) were further characterized. The time course of their expression during GM-CSF stimulation demonstrated a unique pattern for each clone. C15 was expressed on days 0-3, and then decreased markedly. B9 was expressed predominantly on day 3. C9 expression increased gradually to a maximum level on day 7; however, it was also demonstrated in the primitive myeloid line DA3. Tissue specificity was studied. C9 mRNA was identified in a wide variety of murine tissues by Northern blot analysis, while C15 had a relatively restricted pattern of expression, being most prevalent in ovary/uterus, liver and adrenal, in addition to bone marrow. B9 expression appeared limited to bone marrow. Preliminary DNA sequence analysis identified thymosin B-4 (C9) and a lactoferrin-like sequence (C15). B9 appears to be a novel sequence not previously identified. Genomic organization is discussed.

H 129 MEGAKARYOCYTE POLYPLOIDIZATION AND PLATELET FACTOR 4 EXPRESSION, Alexander Nakeff and Edit Hegyi, Division of Hematology and Oncology, Wayne State University School of Medicine,, Detroit, MI 48201

Different ploidy classes of rat megakaryocytes were sorted by flow cytometry (FCM) from highly-purified, perfusion-fixed megakaryocyte cell suspensions prepared by sequential centrifugal elutriation (CE) and percoll gradient centrifugation. The extent of platelet-factor 4 (PF-4) expression in megakaryocytes in different states of maturational development was examined within each ploidy class by the gold labeled antibody detection (GLAD) method using a new anti-PF-4 monoclonal antibody (2E7). The relative number of labeled alpha granules and labeled alpha granule-related small vesicular structures (AGR-SVS) were quantitated in individual megakaryocytes correlated for DNA content and cytoplasmic maturation. We determined that the stage of cytoplasmic maturation exerted a significant effect on the proportion of labeled alpha granules and labeled AGR-SVS. The least mature cells present within each ploidy group exhibited PF-4 labeling mostly in SVS which were not related to alpha granules. During subsequent cytoplasmic maturation, more of the labeled SVS were seen related to alpha granules with more of the alpha granules themselves becoming labeled. Polyploidization also exerted a profound effect on the proportion of the labeled AGR-SVS. SVS are probably Golgi vesicles that are related to lysosomes and/or receptosomes and that play an important role in the intramegakaryocytic transport of PF-4 from the endoplasmic reticulum into alpha granules. These data provide direct evidence of the complexity of megakaryocytic differentiation involving both nuclear endoreduplication and cytoplasmic maturation as reflected in PF-4 expression.

H 130 CHARACTERIZATION OF RECOMBINANT HUMAN GM-CSF PRODUCED BY NAMALWA CELLS, M.Okamoto*, C.Nakayama*, M.Nakai*, H.Yanagi*, M.Namiki**, J.Sakai**, K.Kadota**, M.Fukui**, *Biotechnology Lab., Sumitomo Chemical Co. Ltd., **Res. Lab., Sumitomo Pharmaceuticals, Takarazuka, Hyogo, Japan.

A cDNA coding for hGM-CSF was expressed in human lymphoblastoid Namalwa cells and the secreted hGM-CSF was purified to homogeneity for characterization. We constructed a hGM-CSF expression vector that also encoded mouse DHFR, and transferred it into Namalwa cells. The integrated DNA was amplified by selection in medium containing MTX and cell lines were established which express hGM-CSF at a rate of 10-20 $\mu g/10^6$ cells/day. We purified hGM-CSF from the conditioned media by immunoaffinity, lectin and hydrophobic chromatography. The hGM-CSF showed heterogeneity of molecular mass (16-35kDa) due to the degree of glycosylation. To examine the effect of glycosylation on the function of hGM-CSF, we isolated 2N-(two N-glycosylation sites occupied), 1N-(one site occupied) and 0N-(lacking N-glycosylation)type hGM-CSF. From the results of *in vitro* colony forming assay using human bone marrow cells and of experiment on *in vivo* clearance in rats and monkeys, it was found that addition of N-linked oligosaccharides decreased *in vitro* activity but that dramatically elongated the half life of hGM-CSF in the blood stream.

H 131 REMOVAL OF NATURAL KILLER (NK) CELLS ENHANCES THE RECOVERY OF CFU-S FROM SUBLETHAL DOSES OF TOTAL-BODY IRRADIATION, Klaus Pantel and Alexander Nakeff, Division of Hematology and Oncology, Wayne State University School of Medicine, Detroit, MI 48201

Although the suppression by NK cells and T-cytotoxic/suppressor lymphocytes ($T_{c/s}$) of hematopoietic cell growth in vitro is well-characterized, the paucity of data on the physiologic relevance of this suppression led us to examine the effect of specific depletion of these subsets on the in vivo recovery of CFU-S from a sublethal dose of total-body irradiation (TBI). The number of NK or $T_{c/s}$ cells were depleted in groups of B6D2F1 mice receiving two injections of 0.2 ml ascites containing the monoclonal antibodies NK 1.1 or LYT-2 directed specifically to murine NK cells or $T_{c/s}$ lymphocytes, respectively, at 3 and 2 days prior to a single dose of 9 Gy TBI. Control groups were treated similarly but received either no ascites or control ascites obtained using the fusion partner of the NK 1.1 hybridoma. We measured the femoral content of CFU-S at day 6 post-TBI as an index of early recovery. Pretreatment with NK 1.1-containing ascites resulted in an 8-fold increase over the corresponding values in both control groups. Neither control group differed significantly from the other nor from the group receiving the LYT-2 containing ascites. These results indicate that NK cells may be responsible for the lag in the recovery of CFU-S observed following high doses of TBI and provide the first evidence of the physiologic relevance of NK cells in regulating hematopoiesis.

H 132 HETEROGENEITY IN HUMAN INTERLEUKIN-3 RECEPTORS: A SUBCLASS THAT BINDS HUMAN GM-CSF. Linda S. Park, Della Friend, and David L. Urdal, Immunex Corp., 51 University Street, Seattle, WA 98101

^{125}I-labeled recombinant human IL-3 was used to study the characteristics and distribution of the receptor for IL-3 on human cells. Receptors were found on primary monocytes and leukemia cells from patients with ANL, on some strains of the myelogenous leukemia cell line KG-1, and on cell lines of pre-B or early B cell phenotypes. Binding was rapid at 37°C, while requiring six or more hours to reach equilibrium at 4°C. Equilibrium binding studies with monocytes and the pre-B cell line, JM-1 indicated that IL-3 bound to a single class of high affinity receptor (<500 receptors/cell) with a K_a of approximately 1 x 10^{10} M^{-1}. Examination of a panel of lymphokines and growth hormones revealed that human GM-CSF was able to partially inhibit the binding of ^{125}I-IL-3 to human monocytes, but not JM-1 cells. Additional analysis showed that on KG-1 cells, both human IL-3 and GM-CSF were able to partially compete, with approximately equivalent capacities (on a molar basis) for specific binding of heterologous radiolabeled ligand. This competition occurred at both 37°C (in the presence of sodium azide) and at 4°C and was also observed on many ANL cells. These results can be explained most simply by invoking heterogeneity in the binding sites for IL-3 and GM-CSF in which a subset of receptors bind only IL-3, a subset only GM-CSF, and another subset can bind both, all with high affinity. Evidence for additional heterogeneity was presented by equilibrium binding of ^{125}I-IL-3 and ^{125}I-GM-CSF to KG-1 and ANL cells which consistently produced biphasic Scatchard plots containing both a high and low (10^7 - 10^8 M^{-1}) affinity component.

H 133 A CSF-1 ANTISENSE OLIGODEOXYNUCLEOTIDE INHIBITS PROLIFERATION OF IMMORTALIZED MURINE MONOCYTES: ESTABLISHMENT OF AUTOCRINE REGULATION. Maria C. Birchenall-Roberts[1], Lydia A. Falk[1], Cristina Ferrer[2] and Francis W. Ruscetti[1]. Laboratory of Molecular Immunoregulation, BRMP, DCT, NCI, MD 21701.

Using a combination of v-myc and v-ras oncogenes we have established a growth factor-independent murine monocyte cell line from fetal liver (FL-ras/myc). Biological and molecular characterization of this cell line demonstrated expression of the macrophage growth factor CSF-1 and the c-fms proto-oncogene (CSF-1 receptor). We used two approaches to study the possibility that autocrine regulation may be responsible for the autonomous growth of the FL-ras/myc cell line: (1) use of monoclonal antibodies to bind the CSF-1 protein produced by the cell line, (2) use of antisense oligomers to inhibit the CSF-1 gene products by specific base-pairing of complementary nucleic acids. In this study, we report that using monoclonal antibodies to CSF-1 and antisense oligodeoxynucleotides to CSF-1 mRNA inhibits the in vitro growth of the FL-ras/myc cell line. Incubation of the cell with either the anti-CSF-1 oligomer or the anti-CSF-1 monoclonal antibody decrease cell proliferation by approximately 70%. The combination of oligomer and monoclonal antibody to CSF-1 resulted in 95% inhibition of proliferation. On the other hand, antisense oligomers with 50%-base-pair mismatch with CSF-1 mRNA did not inhibit growth of the FL-ras/myc cell line. These results suggest that CSF-1 production is required for the autonomous growth of the FL-ras/myc cell line. Furthermore, our studies indicate that oligonucleotides can be an efficient tool to block autocrine production of growth factors. The role of the myc and ras oncogenes on expression of CSF-1 and fms genes is being examined.

H 134 EVIDENCE FOR AT LEAST TWO DIFFERENT MECHANISMS REGULATING MACROPHAGE COLONY-STIMULATING FACTOR (M-CSF) IN THE HL-60 PROMYELOCYTIC CELL LINE, Richard A. Sater, Larry D. Cripe and James E. Niedel, Departments of Pharmacology and Medicine, Duke University, Durham, NC 27710.
The ability of various agents to regulate M-CSF expression in HL-60 cells was studied by RNA hybridization. Unstimulated HL-60 cells or cells treated with agents causing granulocytic differentiation (1 μM retinoic acid, 1.3 % dimethyl sulfoxide) do not express M-CSF. However, agents capable of causing monocytic/macrophagic differentiation induce M-CSF expression. Two distinct patterns of message production are observed. After treatment of HL-60 cells with phorbol 12, 13 - dibutyrate (PdBu, 100 nM) or Vitamin D_3 (50 nM) M-CSF expression begins within 16 hours. Addition of the protein synthesis inhibitor, cycloheximide (10 μg/ml), at 0, 4, 8 or 12 hours after treatment with 100 nM PdBu blocks the expression of M-CSF at 24 hours. Calcium ionophore (A23187, 1 μM), tumor necrosis factor (100 U/ml) or gamma-interferon (500 U/ml) lead to M-CSF expression by 2 hours that continues at high levels for at least 24 hours. Addition of cycloheximide (10 μg/ml) one hour before treatment of the HL-60 cells with A23187 does not eliminate the M-CSF message. These findings indicate that M-CSF expression in HL-60 cells induced to differentiate along the monocytic/macrophagic pathway can be regulated by at least two mechanisms, one requiring new protein synthesis and one using existing proteins. Currently, experiments are being performed to identify the DNA sequences in the M-CSF promoter that are necessary for induction of the M-CSF mRNA during monocytic differentiation as well as to identify the nuclear factors controlling these sequences.

H 135 PARTIAL CHARACTERIZATION OF B-CLL CELL-DERIVED CHEMOKINETIC INHIBITORY FACTOR (CIF) TO HUMAN POLYMORPHONUCLEAR LEUKOCYTES, Agneta Siegbahn, Per Venge and Kenneth Nilsson, Departments of Clinical Chemistry and Pathology, University Hospital, S-751 85 Uppsala, Sweden
B-lymphocytic leukemia (B-CLL) cells produce a lymphokine that inhibits migration of human polymorphonuclear leukocytes (PMN). This product, termed chemokinetic ihnibitory factor (CIF) is also produced by a B-CLL cell line immortilized by Epstein-Barr virus and by a subset of normal spleen B-lymphocytes but not by other human cell lines. Purification is in progress and CIF is a heat-labile (56°C for 30 min) glycoprotein with an approximate m.w. of 30 KD. Both granulocyte random and directed migration are inhibited by CIF. The migration inhibition can be totally neutralized by a newly raised antiserum. CIF was present in serum from 64/89 investigated patients with B-CLL. The existence of CIF in serum from these patients contributed to increased susceptibility to bacterial infections.

H 136 THE EFFECTS OF TWO RAT MONOCLONAL ANTIBODIES DIRECTED AGAINST AN IL-3 BINDING PROTEIN ON THE IL-3-INDUCED PROLIFERATION AND G-CSF-INDUCED PROLIFERATION AND DIFFERENTIATION OF 32Dcl3 CELLS, David J. Tweardy, Pamela L. Mott, and Penelope A. Morel, Departments of Medicine and Microbiology, Biochemistry, and Molecular Biology, University of Pittsburgh School of Medicine and the Pittsburgh Cancer Institute, Pittsburgh, PA, 15213.
Information about the interactions of pathways involved in myeloid proliferation and differentiation is critical to our understanding of normal myeloid development and leukemogenesis. We have recently demonstrated that the IL-3 dependent, murine bone marrow-derived cell line, 32Dcl3, undergoes complete differentiation into mature neutrophils when IL-3 is replaced by human G-CSF; differentiation is inhibited by co-cultivation with IL-3. The goal of this research was to examine the effects of the rat monoclonal antibodies, 2F2 and 4G8, directed against a murine IL-3-binding protein, on the IL-3- and G-CSF-induced pathways. In two experiments, 2F2 and 4G8 non-competitively inhibited by up to 50% IL-3-induced proliferation at various concentrations of antibody and IL-3. Antibodies 2F2, in three experiments, and 4G8, in one experiment, each increased G-CSF-induced proliferation by 3- to 5-fold at suboptimal concentrations of G-CSF. In one experiment, co-incubation of 32Dcl3 cells with G-CSF and 2F2 decreased the number of mature neutrophils appearing in culture at 4, 6, 9, and 12 days to 1%, 8%, 21%, and 62%, respectively, compared to 4%, 17%, 60%, and 85%, respectively, for cells cultured in G-CSF alone. These results suggest that binding of 2F2 and 4G8 to 32Dcl3 cells blunts the IL-3-induced proliferation response yet appears capable of triggering partial activation of the IL-3 receptor-mediated pathway and thereby interfering with G-CSF-induced differentiation.

H 137 GM-CSF STIMULATES PROLIFERATION OF MACROPHAGES AND GRANULOCYTE-MACROPHAGE COLONY FORMING CELLS VIA ACTIVATION OF A Na^+/H^+ ANTIPORT, Susan J. Vallance, Nia Cook, C. Peter Downes and Anthony D. Whetton, Department of Biochemistry and Applied Molecular Biology, UMIST, P.O.Box 88, Manchester M60 1QD, U.K.
The biological effects of Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) are now well documented, however very little is known about the intracellular events stimulated by GM-CSF leading to these effects. We have studied the signalling events elicited by GM-CSF in two populations of cells: highly enriched GM-colony-forming-cells and bone marrow derived macrophages. In both of these populations there was no evidence of a direct or indirect role for cyclic AMP or inositol lipid hydrolysis in GM-CSF stimulated cell proliferation. There was also no evidence of increased cytosolic Ca^{2+} levels in cells challenged with this growth factor. However, GM-CSF did stimulate a dose dependent amiloride sensitive increase in intracellular pH in both macrophages and GM-CFC. In macrophages, PAF, which is not a proliferative stimulus, also causes acute activation of the Na^+/H^+ antiport. However, chronic treatment of these cells with either PAF or GM-CSF demonstrates a persistent activation of the pump only in those cells treated with the growth factor, implying that differential mechanisms of regulation of the Na^+/H^+ antiport exist in macrophages. Experiments on the effects of amiloride analogs on GM-CFC and macrophage proliferation also suggest a role for the Na^+/H^+ antiport in GM-CFC and macrophag proliferation. However, it has previously been reported that GM-CSF does not directly activate the Na^+/H^+ antiport in neutrophils. The implications of these observations on the populations employed to study GM-CSF action are discussed.

H 138 FIBROBLAST SUBPOPULATIONS DIFFER IN PRODUCTION OF MYELOID STIMULATORY AND INHIBITORY ACTIVITIES, H. Wang and A.K. Sullivan, McGill Cancer Centre, and Division of Hematology, Royal Victoria Hospital, Montreal, Quebec, Canada.
Previous studies from this laboratory have shown that the predominant population of fibroblastoid cells (FB) grown from rat bone marrow bears surface antigens different from most peripheral organs tested (i.e, lung). Further experiments have indicated that marrow-derived, but not lung-derived cells where stimulated by macrophage conditioned medium (MCM) to secrete growth factors for CFU-C. These cells also produced inhibitors of myeloid colonies. This inhibitory activity had the following characteristics: (1) It was present in low concentration in CM from marrow FB, but increased when ST3+ cells were stimulated by MCM. (2) It was present in high concentration in cultures of lung FB not stimulated by MCM. (3) It appears to be relatively selective for rat myeloid cells, in that it inhibited colony formation of BNML promyelocytic leukemia cells, but not any of eight other rat lines of various tissue origins; it did not inhibit any human leukemic lines tested, or the murine WEHI-3B line. (4) Its activity was absorbed by BNML cells, but not by U937 human monoblastic cells, known to be sensitive to beta-TGF. (5) It inhibited support of CFU-C by both rat spleen cell CM and marrow FB CM.(6) It appears to be different from beta-TGF in that it was acid and heat labile, was not inhibited by neutralizing antibody to beta-TGF, and did not inhibit rat lines shown to be sensitive to the inhibitory effect of beta-TGF. In conclusion, these results show that both rat lung and marrow fibroblastoid cells produce an activity inhibitory to myeloid colonies that appears; this activity appears to be different from other known inhibitors such as beta-TGF.

H 139 HUMAN EMBROYONIC KIDNEY (HEK) CELLS PRODUCE INTERLEUKIN-6 (IL-6), WHICH PROMOTES MEGAKARYOCYTE MATURATION IN-VITRO, Raymond M. Withy, Lori F. Rafield, Joseph P. Winslow, Sara Yankelev, and Cathy L. Oppenheimer, Integrated Genetics, Framingham MA 01701
Polyadenylated RNA isolated from HEK cells was hybridized with a DNA probe encoding IL-6. Three transcripts, 1.3kb, 2.0kb and 3.2kb, were detected, thus indicating the presence of IL-6 specific mRNA in these cells. Western blot analysis of concentrated HEK cell conditioned media using antibody to IL-6 confirmed the presence of IL-6 protein in HEK cell conditioned media. Experiments were then conducted to determine the effect of IL-6 on megakaryocyte maturation in vitro. IL-6 was shown to stimulate megakaryocyte maturation in a single cell anchorage-independent assay, using as the target cells of the assay, a preparation of murine bone marrow which had been depleted of mature megakaryocytes. When HEK cell conditioned media preparations were analysed in this in-vitro assay in the presence of neutralizing antisera to IL-6, a significant proportion of the activity was eliminated. We have previously demonstrated that erythropoietin (EPO) activity, another stimulator of in-vitro megakaryopoiesis, and Transforming Growth Factor Beta (TGF-beta), a potent inhibitor of in-vitro megakaryopoiesis, are also both present in HEK cell conditioned media. Together, these data suggest that previously reported preparations from HEK cell conditioned media, which have in-vitro thrombopoietic activity, may also contain IL-6, EPO and TGF-beta activities. This finding illustrates the problems that can be encountered when assaying for thrombopoietic activity using in-vitro bone marrow assays.

H 140 THE EFFECTS OF SEVERAL GROWTH FACTORS ON THE IN VITRO GROWTH OF MURINE BONE MARROW CFU-F, Norman S. Wolf[1], Qi Ru Wang[2], and Zhao Jie Yan[3], [1]Department of Pathology, University of Washington School of Medicine, Seattle, WA 98195, [2]Experimental Hematology Laboratory, Hunan Medical College, PRC, and [3]163 Hospital, Changsha, Hunan, PRC. The effects of several growth factors on the proliferation of CFU-F were studied. In the present study CFU-F colonies were found to consist of fibroblasts, macrophages and endothelial cells. Growth factors, including IL-3, IL-1α, EGF, FGF, PDGF, GM-CSF, and Buffalo Rat Liver cell-conditioned medium (BRL-CM) were tested for stimulation of the proliferation of CFU-F in a standard culture in both 2% and 15% serum. Overall, the colony numbers produced in 15% serum were much higher than in 2% serum with or without growth factors. However, the influence of several growth factors on CFU-F cultured in 2% serum were relatively greater than in 15% serum, when compared to controls. The stimulation of CFU-F by FGF only occurred in culture with 15% serum, and the stimulation by PDGF only occured with 2% serum. Overall, the strongest stimulations were produced by PDGF, IL-3, and BRL-CM. Combining the other growth factors with IL-3, PDGF or IL-1α enhanced their effects only modestly. The stimulation by growth factors included increases of the cell numbers between and within colonies as well as an increase in the number of colonies. When GM-CSF was added to the cultures, no CFU-F colonies were seen. The study produced results which suggest a complex interaction mediated by growth factors between fibroblasts and other stromal cells within the CFU-F colonies and within the bone marrow itself.

H 141 MACROPHAGE INFLAMMATORY PROTEIN-1 IS A NOVEL COLONY STIMULATING FACTOR S.D. Wolpe, J. Gianotti, B. Sherry, G. Davatelis, A.Cerami and S. Clark. Genetics Institute, Cambridge, MA and Rockefeller University, New York, NY

Murine macrophages secrete two novel heparin-binding proteins when stimulated with endotoxin. Macrophage inflammatory protein-1 (MIP-1) is a doublet of 8000 daltons which is mildly chemotactic for human PMN's and induces them to secrete hydrogen peroxide; in addition, when administered subcutaneously into the footpad of a mouse, MIP-1 induces a localized inflammatory reaction (JEM 167:570, 1988; JEM 167:1939, 1988 and JEM (in press). MIP-2 is a single chain of approximately 6000 daltons which also binds heparin (PNAS, in press). MIP-2 is extremely chemotactic for human PMN's but does not induce an oxidative burst. Both proteins are members of a superfamily of inflammatory mediators which have recently been described (reviewed in Wolpe and Cerami, submitted).

In the present communication we demonstrate that MIP-1, but not MIP-2, is capable of inducing colony formation in a murine bone marrow assay. At a concentration of 1 ug/ml MIP-1 induced an average of 14.8 colonies compared to a control value of 2.5; significant increase in colony formation was seen at concentrations of 200 ng/ml as well.

Murine MIP-1 was not active in a human bone marrow colony assay nor did it increase proliferation in any of several IL-6, GM-CSF or IL-3 responsive cell lines. MIP-2, purified under similar conditions, exhibited no significant increase in colony formation. Further, specific polyclonal rabbit antiserum to MIP-1 abolished the induction of colonies in a dose-dependant manner.

Experiments are under way to see if MIP-1 acts directly or indirectly on progenitors.

H 142 REGULATION OF EOSINOPHIL PROGENITOR CELLS (CFU-EO) DURING PARASITE-INDUCED EOSINOPHILIA, Karen M. Young, Department of Pathobiological Sciences, University of Wisconsin-Madison School of Veterinary Medicine, Madison, WI 53706

A murine model of toxocariasis is being utilized to study the regulation of eosinophil progenitor cells. C57Bl/6 mice (Jackson Lab.) infected with embryonated eggs of Toxocara canis develop peripheral blood eosinophilia by 7 days that persists through 28 days post-infection. Bone marrow eosinophils are increased at 2 days, peak at 7 days, and remain increased at 21 days post-infection. IL 3-responsive bone marrow CFU-EO, assayed in a soft agar system using WEHI 3-conditioned medium as the source of IL 3, are significantly decreased in frequency on days 2 (70% of control) and 3 (40% of control) as compared to uninfected controls. On days 7 through 21 no difference in CFU-EO frequency can be detected. These data suggest that eosinophil production early in the course of parasite infection is mediated by maturation of CFU-EO and proliferation of precursors rather than by increased commitment of stem cells to the eosinophil lineage. Spleen cells from parasitized mice elaborate eosinophilopoietic factors in response to Toxocara excretory-secretory antigens (TEX), but not in response to Trichinella antigens. Supernatants from TEX-stimulated spleen cells support the growth of CFU-EO and the proliferation of the IL 3-dependent cell line DA-1, indicating the presence of IL 3. In addition, these supernatants enhance the proliferation and differentiation of eosinophils in liquid bone marrow culture and potentiate the capacity of WEHI cell-derived IL 3 to support the growth of CFU-EO, suggesting the presence of IL 5. It appears that both IL 3 and IL 5 are elaborated in response to parasitic infection. Their interrelationship in regulating eosinophilopoiesis remains to be elucidated.

H 143 SPECIFIC POTENTIATION OF ERYTHROID PROGENITORS *IN VITRO* BY ACTIVIN, John Yu, Li-en Shao, Joan Vaughan, Wylie Vale and Alice L. Yu, Research Institute of Scripps Clinic, The Salk Institute for Biological Studies, and University of California at San Diego, La Jolla, CA 92037

We had recently demonstrated a regulatory effect of activin, a TGFβ-like protein, on human erythropoiesis (Yu *et al.*, *Nature* 330:756, 1987; "Characterization of the potentiation effect of activin on human erythroid colony formation *in vitro*," *Blood*, in press, 1988). It was found that activin and inhibin are two related molecules with different subunit associations and functionally opposite activities. DNA synthesis of erythroid progenitors was specifically stimulated by activin. The potential effect of colony formation by activin is restricted to progenitor cells such as BFU-E and CFU-E, but not other more mature erythroid cells such as cluster-forming cells. In addition, the effect of activin is restricted to cells of the erythroid lineage, with no effect on other cell types. Activin requires the presence of both monocytes and T lymphocytes to exert its effect. Similar potentiation by activin was also found in serum-free bone marrow cultures.

Late Addition

H 144 MOLECULAR CHARACTERIZATION OF THE HUMAN G-CSF RECEPTOR, Belinda R. Avalos, Judith C. Gasson, Laurine G. Connors, David W. Golde, Lawrence M. Souza, and Dennis J. Slamon, Division of Hematology-Oncology, Ohio State University, Columbus, OH 43210; Division of Hematology-Oncology, UCLA School of Medicine, Los Angeles, CA 90024; and AMGEN, Thousand Oaks, CA 91320. Human granulocyte colony-stimulating factor (G-CSF) is a multifunctional monokine that stimulates proliferation of various hematopoietic and non-hematopoietic cells, and serves as an important regulator of the growth, differentiation, and function of neutrophilic granulocytes. Using a biologically active modified biosynthetic human G-CSF (G-CSF-lys) that was radioiodinated to high specific activity, the molecular and biochemical characteristics of the human G-CSF receptor were identified. A single class of high affinity receptors (Kd 50-400 pM, 37-736 sites/cell) was found on peripheral blood neutrophils, the myeloid leukemic cell lines KG-1 and HL-60, and the small cell carcinoma line H128. Chemical cross-linking identified the G-CSF receptor as a single subunit protein of M_r 150,000. The basic structure of this receptor was identical on both normal and malignant hematopoietic cells, as well as non-hematopoietic cells. Specific binding of ^{125}I-G-CSF-lys to purified neutrophil membranes suggests localization of the human G-CSF receptor to the cell membrane.

Receptors; Cellular Interactions

H 200 PREPARATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST THE MURINE IL-4 RECEPTOR. M. Patricia Beckmann, Kenneth Schooley, Tim Vandenbos, Della Friend, Ric Raunio, Byron Gallis, Alan Alpert, David L. Urdal, Paul Baker, and Linda S. Park. Immunex Corp., 51 University Street, Seattle, WA 98101.

Interleukin-4 (IL-4, also known as B-cell stimulating factor-1 or BSF-1) is a cytokine which was initially shown to induce B-lymphocyte proliferation, to increase surface Ia expression, and to induce some activated B cells to differentiate into IgE secreting cells. IL-4 binds specifically to a cell surface receptor on cells from a variety of lineages including B-cells and T-cells. In general both primary cells and in vitro cell lines express < 5,000 IL-4 receptors per cell. We have prepared monoclonal antibodies against the murine IL-4 receptor by injecting a subclone of the cytotoxic T-cell line CTLL expressing high IL-4 receptor levels into rats. Following subsequent booster injections of IL-4 receptor bearing cells, rat-mouse hybridomas were prepared. Primary screening of hybridomas utilized radioiodinated partially purified murine IL-4 receptor. Out of approximately 400 hybrids screened, two cell lines were selected for further characterization. Preliminary evidence indicates that both monoclonals are capable of immunoprecipitating labeled IL-4 receptor protein, and that one antibody is able to inhibit ^{125}I-IL-4 binding to intact cells. Further characterization of these antibodies is underway and will be presented.

H 201 MOLECULAR CHARACTERIZATION OF A CHROMOSOMAL TRANSLOCATION IN A STEM CELL LINE, C.G. Begley, P.D. Aplan, M.P. Davey, K. Nakahara, K. Tschorz, J. Kurtzberg, B. Haynes, D. Cohen, T.A. Waldmann, I.R, Kirsch, Metabolism Branch, NCI and NCI-Navy Medical Oncology Branch, Naval Hospital, Bethesda, MD 20814

A CD7-positive, CD3-,CD4-,CD8-negative "T cell" line with a translocation between chromosomes 1 and 14 (1;14)(p33;q11) and a deletion on chromosome 1, del(1)(p33), was examined. The cell line was able to differentiate to myeloid cells in response to differentiating agents. A genomic DNA library was prepared and the germline and rearranged alleles involved in the translocation were cloned, mapped, and sequenced. Chromosomal in situ hybridization confirmed the origin of partners in the reciprocal exchange. The del 1p33 was also cloned. It mapped to within 10 kb of the region affected by the translocation. A rearrangement between the delta rec element and a pseudo J alpha gene that served to delete the delta locus was also identified on the normal chromosome 14. We are currently exploring the causes and consequences of this translocation with reference to stem cell differentiation.

H 202 CHRONIC SCHISTOSOMIASIS MANSONI: SYSTEMIC HYPERPLASIA AND LONG-TERM PROLIFERATION OF MONO-MACROPHAGIC CELL LINEAGE SUSTAINED BY MEDULLAR AND PERIPHERIC MYELOPOIETIC STROMAS. Radovan Borojevic, Márcia C. El-Cheikh, Hélio S. Dutra. Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro, 21941 Rio de Janeiro, Brazil. - In chronic schistosomiasis, blood monocytosis and hyperplasia of mono-macrophagic cell lineage involve liver, spleen and periovular granulomatous reactions. Total number of bone marrow myeloid precursors and their proliferation stimulated with GM-CSF were similar in normal and infected mice. Differentiation of GM committed stem cells was deflected in favor of macrophagic lineage with concomitant delay of granulocyte differentiation. Peripheric proliferation of macrophages was observed in granulomas. Macrophages isolated from granulomas secreted a considerable M-CSF activity. Myeloid stroma established from infected-mice bone marrow and in vitro cultures of whole periovular granulomas, isolated from infected-mice livers, sustained spontaneously growing foci of monocytoid cells. Normal myeloid stroma had not this activity. Connective stroma established from schistosomal granulomas sustained in Dexter-type cultures a long-term production of monocytoid cells that proliferated in soft-agar cultures when stimulated with GM-CSF. These cells differentiated exclusively into macrophages, both in soft-agar and in liquid cultures. Skin fibroblasts from the same mice did not sustain this proliferation. Periovular granulomas in chronic schistosomiasis function as autonomous sites of a long-term macrophage proliferation, containing specific myelopoietic stroma and an internal source of M-CSF activity.

(Supported by UNDP/World Bank/WHO, FINEP and CNPq)

H 203 NATURAL HISTORY OF ERYTHROPOIETIN (EPO)-BINDING. Samuel H. Boyer and Katherine T. Landschulz, Department of Medicine, The Johns Hopkins Univeristy School of Medicine, Baltimore, MD 21205 Epo response/binding was asssayed in purified murine CFUe and their descendents. Six features emerged: First, EPO-binding is in rapid flux: half-time for internalization is ~4 min at 37°. Second, based on 48h colony formation following EPO addition or subtraction during culture, most CFUe require EPO only during the first 8h. Third, when stripped of EPO acquired in vivo and then assayed for ^{125}I-EPO-binding, CFUe display two classes of binding sites: ~40% with 95pM dissociation constants (K_d) and 60% with 655pM K_d. Fourth, EPO acquired in vivo occupies > 75 high-affinity (95pM) sites/cell when CFUe are first harvested. Although such occupancy exceeds by > 10x bound EPO needed for colony formation, no CFUe mature into colonies without added EPO. The inference is that repeated occupancy of rapidly turning-over EPO-receptors is required for CFUe maturation. Fifth, total EPO-binding sites fall to 40% zero-time levels after 16h in EPO-supplemented culture and disappear after 28h culture. Scatchard analyses indicate that 95pM binding-sites vanish by 8h but 655pM sites are only slightly diminished. Such disappearance of one class of site and the persistence of the other suggests that (a) at least two gene-products mediate EPO-binding, (b) high-affinity (95pM) sites mediate the growth function of EPO, and (c) because they linger after growth is assured, low-affinity (655pM) sites may mediate some other EPO function. Sixth, because the relative abundance of 105 and 90kDa polypeptides found on CFUe cross-linked with ^{125}I-EPO is not correlated with affinity site abundance, we suppose that either the two classes occupy different but like-size receptor proteins or that high-affinity is modulated by a modifier which disappears after 8h culture.

H 204 A T-LYMPHOID CELL LINE RESPONDS TO A THYMIC STROMAL CELL LINE BY EXPRESSION OF THYMOCYTE ANTIGENS, B. Kay Brightman[1], K. George Chandy[2], Robert H. Spencer[2], and Hung Fan[1], [1]Department of Molecular Biology and Biochemistry and [2]Department of Medicine, College of Medicine, University of California, Irvine, CA 92717

We have cloned T-lymphoid and stromal lines from a murine thymic tumor induced by a retrovirus carrying the v-myc oncogene. The T-lymphoid line was initially negative for Thy 1.2 and CD4 thymocyte differentiation antigens. Cocultivation of the Thy 1.2^- $CD4^-$ line with the stromal cell line resulted in sequential expression of Thy 1.2 and CD4. Moreover, cocultivation with the stromal line was necessary for the appearance of Thy 1.2^+ $CD4^+$ cells. These cells may provide a model system for studying thymocyte-stromal cell interactions in T-lymphoid differentiation.

H 205 INTERLEUKIN 2 ACTIVATES A TYROSINE PROTEIN KINASE THROUGH THE 75KD RECEPTOR ON HUMAN LEUKEMIA CELL LINES, John E. Casnellie & Elizabeth M. Saltzman, Department of Pharmacology and Cancer Center, University of Rochester Medical Center, Rochester, N.Y. 14642. We have previously shown that interleukin 2 (IL-2) stimulation of T cells results in an increase of tyrosine phosphorylations of several proteins (J. Biol. Chem.(1988) 263, 6956) The phosphorylations were observed in IL-2-dependent cells at concentrations of IL-2 specific for binding to the high-affinity receptor. The high-affinity receptor for IL-2 consists of two different IL-2-binding proteins with molecular weights of 55,000 (p55) and 75,000 (p75). The p75 receptor, independent of p55, appears to be responsible for IL-2-induced signal transduction that results in proliferation. If activation of a tyrosine protein kinase is essential to the mechanism of IL-2-induced signal transduction then the p75 receptor alone would expected to activate a tyrosine protein kinase in response to IL-2-stimulation. We assessed the ability of IL-2 to induce tyrosine protein kinase activation via p75 by studying this response in the leukemic cell lines Hut 78 and YT. These cells have been found to express p75 as the predominant receptor for IL-2. Treatment of these cells with IL-2 resulted in an increase in tyrosine phosphorylation of proteins with molecular weights identical to that previously observed in IL-2-dependent cells where the signal transduction occurs through the high affinity-receptor. The concentrations of IL-2 required to induce these phosphorylations were the same as required for binding to the isolated p75 receptor. The phosphorylations were not affected by antibodies that inhibit binding of IL-2 to the p55. These results indicate that the signaling capacity for IL-2-induced increase in tyrosine protein kinase activity resides in the p75 receptor.

H 206 DISTINCT CONTROLS OF TRANSFERRIN RECEPTOR EXPRESSION IN ERYTHROID AND NON-ERYTHROID CELLS, Roxanne Chan, Herbert M. Schulman and Prem Ponka, Lady Davis Institute, Jewish General Hospital and Dept. of Medicine, McGill University, Montreal, Quebec, Canada. Transferrin receptors (TfR) are necessary for transferrin-mediated cellular iron uptake in all mammalian cells. The receptors are especially abundant in developing erythroid cells which are actively synthesizing hemoglobin, and in proliferating non-erythroid cells where iron is essential for ribonucleotide reductase, a rate-limiting enzyme for DNA synthesis. Neither the induction mechanism nor the control of the receptors in these two cell classes is understood. However, while receptor numbers are positively correlated with proliferation in non-erythroid cells, in erythroid cells their numbers increase during differentiation and, therefore, are negatively correlated with proliferation. The aim of this study was to explore the possibility that there is an erythroid-specific regulatory mechanism for the TfR. Transferrin receptors, measured by ^{125}I-transferrin binding, were determined in uninduced or DMSO-induced Friend murine erythroleukemia cells under conditions which are known to affect their expression in proliferating cells. Hemin lowered the TfR levels in both the induced and uninduced Friend cells suggesting that the erythroid and non-erythroid TfR expression are under similar negative feedback control. However, as compared to the uninduced Friend cells, the cells induced into erythroid differentiation did not respond to either DNA synthesis inhibitors (e.g. hydroxyurea) or iron chelating agents (desferrioxamine or 2,2'-dipyridyl) which both stimulate TfR expression in proliferating non-erythroid cells. The lack of responsiveness to these stimuli suggests that there is a distinct regulatory mechanism for TfR expression during erythropoiesis.

H 207 TRANSMODULATION OF M-CSF RECEPTORS BY GM-CSF ON MURINE PERITONEAL EXUDATE MACROPHAGES IS REGULATED TRANSCRIPTIONALLY: FAILURE OF COUPLING BETWEEN GM-CSF AND M-CSF RECEPTORS IS ASSOCIATED WITH LEUKEMOGENESIS. Ben Chen, Mark Mueller and Nelleke Barendsen. Div. of Hematology and Oncology, Department of Internal Medicine, Wayne State University, Detroit, MI 48201. Normal murine peritoneal exudate macrophages (PEM) display both M-CSF and GM-CSF receptors and can be induced by either one of them to undergo clonal growth and macrophage colony formation in vitro. The proliferative response of PEM to M-CSF was greatly enhanced by the addition of a subliminal concentration of GM-CSF. J774, an established factor-independent monocytic tumor cell line, also expresses both types of CSF receptors and responds to them as well with an enhanced proliferative rate. No synergistic response, however, was observed in J774 cells when both CSFs were added in cultures. Treatment of GM-CSF caused a transient down-regulation of M-CSF receptors in normal PEM but not in J774 cells. On the contrary, treatment with phorbol ester tumor promoter (TPA) induces M-CSF receptor down-regulation in both populations. Northern blot analysis using c-fms probe indicates that transmodulation of M-CSF receptors by GM-CSF and TPA is regulated at transcriptional level. Since protein kinase C (PK-C) has been identified as the binding site of phorbol ester, this finding implies that these two types of receptors are very likely linked by the PK-C system through which signal transduction by various types of CSF receptors are coordinated. The fact that TPA but not GM-CSF down-regulates M-CSF receptors and c-fms expression in J774 cells further suggests that the coupling between GM-CSF receptors and PK-C system are broken which may lead to the ontogeny of leukemogenesis and differentiation arrest in J774 cells.

H 208 CHARACTERIZATION AND MOLECULAR FEATURES OF THE CELL SURFACERECEPTOR FOR HUMAN GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR, Shigeru Chiba, Arinobu Tojo, Toshio Kitamura, Kohei Miyazono, Akio Urabe and Fumimaro Takaku, The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.

The receptors for human GM-CSF on the surfaces of normal and leukemic myeloid cells were characterized using ^{125}I-labeled bacterially synthesized GM-CSF. Scatchard analysis of the ^{125}I-GM-CSF binding to peripheral blood monocytes and two GM-CSF-responsive myeloid cell lines (U-937 and TF-1) demonstrated two classes of binding site: one with high affinity (Kd_1 = 10-40 pM) and the other with low affinity (Kd_2 = 0.9-2 nM). For U-937 cells, the number of low-affinity receptors was as many as 10,800 sites/cell. The result of cross-linking study indicated that M_r values of 135,000, 100,000 and 80,000 were the individual components of the human GM-CSF receptor. In the experiment usung U-937 cells, GM-CSF appeared to bind preferentially to the M_r 135,000 species among the three receptor molecules. These results suggest that the human GM-CSF receptor possibly forms a multi-chain complex.

H 209 THE RECEPTOR BINDING SITE OF RECOMBINANT HUMAN ERYTHROPOIETIN MAPPED WITH MONOCLONAL ANTIBODIES, Alan D'Andrea, Harvey Lodish, Edward Alderman, The Whitehead Institute for Biomedical Research, Department of Biology, Massachusetts Institute of Technology, and Genetics Institute, Cambridge, MA 02140

We have generated four high affinity monoclonal antibodies (MoAbs) to recombinant human erythropoietin (EPO). Two MoAbs, designated Group I, bind to an epitope within the N-terminal 20 amino acids of EPO. Two other MoAbs (Group II) do not bind to any of the eight tryptic fragments of EPO. We used murine erythroleukemia (MEL) cells to test the MoAbs for inhibition of EPO-receptor binding. MEL cells express 277 high affinity receptors for EPO per cell (Kd = 0.67). Two putative EPO receptor polypeptides (M.W. = 100 kDa and 85 kDa) were revealed when radiolabeled EPO was bound to the surface of MEL cells, followed by crosslinking with disuccinimidyl suberate. To assay our MoAbs, MEL cells were incubated with radioiodinated EPO in the presence or abscence of unlabeled EPO; radioiodinated EPO was pre-incubated with various concentrations of the MoAbs. Group I MoAbs do not inhibit binding of EPO to the MEL EPO-receptor, but Group II MoAbs do inhibit binding in a dose dependent manner. We conclude that the amino terminus of EPO is not involved in EPO-receptor binding and suggest that Group II MoAbs recognize the EPO-receptor binding site.

H 210 CHRONIC SCHISTOSOMIASIS MANSONI: EXTRAMEDULLAR PROLIFERATION OF EOSINOPHIL GRANULOCYTES. Márcia C. El-Cheikh, Hélio S. Dutra, Radovan Borojevic. Departamento de Bioquímica, Instituto de Química, Universidade Federal do Rio de Janeiro, 21941 Rio de Janeiro, Brazil. - Chronic schistosomiasis mansoni is characterized by a tenfold increase of eosinophil precursors and a small increase of mature eosinophils in the bone marrow. It is concomitant with a moderate blood eosinophilia, with apearance of circulating immature eosinophils. Proliferation of eosinophils in inflammed tissues in chronic schistosomiasis has been described, and in previous research we have demonstrated induction of eosinophil myelopoiesis on intraperitoneal glass implants. This eosinopoiesis has been shown to depend upon mobilized macrophages (Borojevic et al. Exptl. Parasitol. 62: 349-355. 1986). - In the present study we cultivated in vitro non-adherent bone marrow cells in the presence of supernatants of cells mobilized onto the intraperitoneal glass implants done in normal and in schistosome-infected mice. Cell proliferation was monitored in the liquid phase of the culture and among cells attached to the substrate. Myeloid clusters composed exclusively of eosinophils and their precursors were observed on the substrate, from the first day of culture on. A peak of eosinophil production was observed on the second and the third days. Supernatants of implants done in normal mice had only a low stimulatory activity for eosinophil proliferation, and no eosinophils were observed from the second day on in control cultures. In chronic schistosomiasis, intense stimulation of medullar eosinopoiesis is concomitant with accelerated release of eosinophils to the circulation. Immature eosinophils mobilized in tissue infiltrates are under local stimulation of proliferation, resulting in terminal amplification of eosinophil populations.

(Supported by UNDP/World Bank/WHO, FINEP and CNPq)

H 211 GENETIC ANALYSIS OF REGULATION OF HUMAN GM-CSF GENE EXPRESSION IN PRIMARY T LYMPHOCYTES, John Fraser, Stephen D. Nimer, and Judith C. Gasson, Division of Hematology-Oncology, Department of Medicine, UCLA School of Medicine, Los Angeles, CA 90024-1678

Human granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates the proliferation and maturation of myeloid progenitor cells, and enhances the functional activity of mature neutrophils, monocytes, and eosinophils. Expression of GM-CSF is tightly regulated, and is limited to activated T lymphocytes and certain cytokine-activated fibroblast, endothelial and macrophage cell types. We have previously used T-lymphoblast cell lines to identify a strong, inducible promoter activity associated with a small region of DNA (90 bp) immediately upstream of the cap site. Furthermore, nuclear extracts prepared from these cells protect this region in DNase I footprinting studies. We now present data which extend this analysis from cell lines to primary human T lymphocytes. Human T lymphocytes purified by density centrifugation and adherence are stimulated for 48 hours with 1% phytohemagglutinin (PHA), electroporated with GM-CSF promoter/chloramphenicol acetyltransferase (CAT) constructs, and cultured in medium supplemented with 1% PHA for a further 24 hours. The results show that 660 bp of sequence upstream of the cap site promotes transcription of the CAT gene in PHA-stimulated T lymphocytes. As with cell lines, deletion of all but 90 bp of sequence immediately upstream of the cap site had no significant effect on promoter activity. We have now produced a family of small deletions from within the footprint region, and show that deletion of as little as 18 bp abolishes promoter activity in these cells. This work provides the basis for more detailed analysis employing site-directed mutations to precisely define the sequences and mechanisms by which GM-CSF expression is regulated.

H 212 CHARACTERIZATION OF CELLULAR RECEPTORS FOR ERYTHROID DIFFERENTIATION FACTOR. Masayuki Hino, Arinobu Tojo, Kohei Miyazono, Akio Urabe, and Fumimaro Takaku, The Third Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

Erythroid differentiation factor(EDF), also named activin A, is a polypeptide which induces differentiation of a murine Friend erythroleukemia cell clone F5-5. It is composed of two inhibin βA chains and is one of the members of transforming growth factor β(TGF-β) family. We have examined the binding of EDF to F5-5 cells. ^{125}I-EDF bound to F5-5 cells in a time- and temperature-dependent, specific, saturable, and reversible process. The binding of ^{125}I-EDF to F5-5 cells reached maximal after 60 minutes at 15°C. TGF-β1 had no significant effect on the differentiation of F5-5 cells and the binding of EDF to F5-5 cells. Scatchard analysis of the equilibrium binding data revealed that F5-5 cells had a single class of EDF receptor and 6000 binding sites per cell with an apparent Kd of 800pM. Affinity cross-linking experiments with DSS demonstrated three radiolabeled components of 140kDa, 76kDa, and 67kDa under both reducing and nonreducing conditions, which disappeared in the presence of excess unlabeled EDF, implying Mrs of 115,000, 51,000, and 42,000 for EDF receptor.

H 213 QUANTITATION OF MURINE ERYTHROID PROGENITOR CELL GROWTH IN VITRO BY USE OF A RAPID SPECTROPHOTOMETRIC ASSAY FOR HEMOGLOBIN

Kenneth G. Jones and Betty A. Wong, Pharmaceutical Research Department, Robert Wood Johnson Pharmaceutical Research Institute, Toronto, 19 Green Belt Drive, Don Mills, Ontario, Canada M3C 1L9.

A modified benzidine peroxide method which employed 3,3',5,5'-tetramethyl-benzidine was utilized to measure hemoglobin synthesis induced by recombinant erythropoietin (EPO) in a methylcellulose culture system, using mouse bone marrow cells. The quantitation of hemoglobin content in the cultures correlated well with the number of erythroid clonogenic progenitor cells. This method was sufficiently sensitive to allow for hemoglobin measurement in 2 to 3 murine erythroid bursts. At plateau concentrations of EPO (CFU-E 0.3 - 0.6 units/ml, BFU-E > 0.6 units/ml), the number of erythroid colonies and the amount of hemoglobin synthesized was directly proportional to the number of bone marrow cells plated. At any given EPO concentration, the CFU-E colony count correlated well with the day 2 or day 3 hemoglobin content. However, the EPO dose-response curve for BFU-E was dissimilar to the 9-11 day hemoglobin curve. No plateau for hemoglobin was reached at concentrations as high as 5 units/ml. The number of CFU-E's which developed in the presence of 0.6 units/ml of EPO was 9- to 18-fold greater than without EPO. The benzidine peroxide hemoglobin assay provided an accurate reflection of the activity of recombinant erythropoietin in the murine erythroid cell culture system.

H 214 ENGRAFTMENT OF IMMUNE-DEFICIENT MICE WITH HUMAN HEMATOPOIETIC STEM CELLS.

Suzanne Kamel-Reid and John E. Dick, Department of Genetics, The Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8. In the mouse we have much information about the cells that comprise the stem cell hierarchy and the events that regulate this complex cell system because of the availability of both in vivo and in vitro assays for various stem and progenitor cells. In contrast there are no in vivo assay systems for human pluripotent stem cells. As a prelude to such an in vivo assay for human stem cells, we have engrafted immune deficient mice with normal human bone marrow. The recipient animals are triple recessive for beige/nude/xid and therefore deficient not only in T and B cells but also NK and LAK activity. Mice were given sub-lethal doses of irradiation and infused with 7-10x10^6 human bone marrow cells. Southern analysis of DNA taken from mouse bone marrow and spleen 14 days after infusion indicates that approximately 0.3-3% of the cells present were human. At various times after infusion of human bone marrow, the animals were sacrificed and the bone marrow and spleen were plated in in vitro colony assays that were only able to support human progenitors. Significant levels of macrophage, granulocyte and erythroid progenitors were detected in these tissues indicating seeding, proliferation and differentiation of human myeloid progenitors. There was a rapid increase in human progenitors within the first 14 days of engraftment and this level of engraftment was maintained for as long as five weeks with no evidence of decline. The human identity of these progenitors was established by human growth factor dependence and molecular detection of human DNA sequences. The fact that human in vitro progenitors have little if any self-renewal potential and the fact that progenitor cells were continuously produced for at least as long as five weeks of reconstitution suggests that early progenitor or stem cells have engrafted these animals. Engraftment of these animals was not dependent on exogenously applied human GM-CSF or human IL-3. These data lay the foundation for the development of animal models for a wide variety of human hemopathies including leukemia and human infectious diseases.

H 215 IMMATURE HUMAN ERYTHROID CELLS EXPRESS MOLECULES RELATED TO LYMPHOCYTE HOMING RECEPTORS.

Geoffrey S. Kansas and Morris O. Dailey, Department of Pathology, University of Iowa College of Medicine, Iowa City, Iowa 52242.

We have previously demonstrated that the anti-lymphocyte homing receptor mAb 515 recognizes a broadly dispersed family of glycoproteins expressed on all leukocytes, fibroblasts and epidermal keratinocytes, but absent from platelets and mature erythrocytes. We report here that normal immature human erythroid cells express molecules recognized by 515. Flow cytometry analysis of low density bone marrow cells (LDBMC) revealed a trimodal distribution of 515 staining on glycophorin$^+$ LDBMC. Interestingly, this pattern of 515 expression on erythroid cells was inversely correlated with differentiation; the least mature committed erythroid cells express high levels of 515-defined structures, and 515 staining declined in a discrete fashion as the cells progressed through phenotypically defined stages of erythroid development. Collectively, these data suggest that 515 may identify adhesion structures involved in erythropoiesis.

H 216 CHARACTERIZATION OF HUMAN IL-3 RECEPTORS ON A MULTI-FACTOR DEPENDENT CELL LINE TF-1. Tomoaki Kuwaki, Toshio Kitamura, *Shigeru Matsuki, Arinobu Tojo, Kohei Miyazono, Akio Urabe and Fumimaro Takaku. The Third Department of Internal Medicine, Faculty of Medicine,University of Tokyo, Tokyo, Japan. *Pharmaceutical Laboratory, Kirin Brewery Co.LTD. Maebashi, Gumma, Japan.

Human interleukin-3(IL-3) is a lymphokine that stimulates the proliferation and differentiation of pluripotent hemopoietic stem cells. We have examined the receptors expressed on a multi-factor dependent human cell line TF-1 established from bone marrow cells of a patient with erythroleukemia. Human recombinant IL-3 expressed by E.coli stimulates the growth of TF-1 cells in a dose-dependent manner. IL-3 was radioiodinated to high specific activity with the Bolton-Hunter reagent. Specific binding of ^{125}I-IL-3 to TF-1 cells were observed at 15°C for 2hr. Scatchard analysis demonstrated the existence of a single class of IL-3 receptor on TF-1 cells and 1000 binding sites per cells. Chemical cross-linking studies with DSS demonstrated two radiolabeled components with molecular masses of 80kDa and 155kDa, which implies 65kDa and 140kDa molecules for human IL-3 receptors.

H 217 THE ROLE OF HOMEOBOX GENES IN HUMAN HEMATOPOIESIS. P. Lowney, W.-F. Shen, T. A. Simonitch, F. M. Hack, H. J. Lawrence, and C. Largman. VA Medical Center, Martinez, CA, Dept of Internal Medicine, UC Davis, Davis, CA

We hypothesize that commitment and differentiation of hematopoietic stem cells involves homeobox genes. Expression of Hox 2.1 and 2.2 homeobox genes is restricted to HEL cells (erythroid lineage). Hox 2.3 and 2.6 genes are also expressed in a restricted pattern: present in the erythroid HEL and K562 lines, absent in the myeloid lines U937, KG1, and HL60; and present in some T and B cell lines. Screening of a monocyte cell line (U937) cDNA library has yielded a new 2.3 kb homeobox-containing cDNA (PL-1). Comparison with published homeobox sequences shows that PL-1 is most closely related to the Hox 2.3 homeobox, and unrelated to all flanking region sequences. The PL-1 gene is expressed in a restricted pattern: present in the monocytic lines U-937 and KG-1, partially present in HEL cells (partial moncytic potential); absent in K-562, HL-60, and two B cell lines; and present in 1 of 4 T cell lines (Molt 3). Reduced stringency screening of Northern gels reveal additional homeobox genes in T and B cells, and in HL-60 cells. Erythroid differentiation of HEL and K562 with hemin yields increased Hox 2.6 mRNA; induction of erythroid differentiation in HEL with AraC results in increased Hox 2.1, 2.2, 2.3, 2.6 mRNA levels; Ara C induced differentiation of K562 cells results in selective dimunition of one of two Hox 2.3 mRNA transcripts. Lineage restricted expression of homeobox genes for human erythroid, monocytic, promyelocytic, and lymphoid cell lines, as well as changes in homeobox gene transcripts following cell differentiation suggests that these genes play a role in hematopoiesis.

H 218 ADENOSINE DEAMINASE EXPRESSION VECTOR ANALYSIS. K.A. Moore, F.A. Fletcher, D.K. Villalon, D.H. Hawkins and J.W. Belmont. Howard Hughes Medical Institute, Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX 77030.

Human adenosine deaminase (hADA) deficiency is being evaluated as a model system for gene therapy utilizing bone marrow transplantation. We have developed recombinant retroviral vectors to deliver and express hADA in a murine model system. The pΔNN2ADA vector (derived by substituting the *neo* gene with hADA cDNA in the parental N2 vector) was established in the GP+E-86 packaging cell line. Since expression analysis of gene transfer experiments in animal models is sometimes complicated by endogenous expression of the same gene, we have developed and characterized a monospecific affinity purified antibody to hADA. The polyclonal rabbit antibody was raised against a synthetic oligopeptide derived from the terminal 15 C'amino acid sequence. Normal mouse ADA does not contain the 11 terminal C' amino acids. Immunohistochemical staining with the anti-hADA (α-hADA) antibody has determined the titer of this virus to be 10^6 infectious units/ml. In bone marrow transplant experiments, day 14 CFU-S demonstrated an infection efficiency of 25% (12/48) by both Southern and Western blot analysis. No infection events without corresponding expression of hADA were detected. All reconstituted recipients (32/32) express hADA in their peripheral blood at 3 weeks after transplant. The GP+E-86 pΔNN2ADA cell line is helper free by marker rescue assay and has remained live virus free after multiple passages. In addition, no live virus was detected in the plasma of reconstituted animals at 3 and 9 weeks post transplant. Expression in long term recipients is being determined by double immunofluorescent labeling of discrete hemopoietic cellular subsets, using cell surface antigen markers and detection of hADA in the cytoplasm by α-hADA. The labeled cells are then analyzed by flow cytometry to assess percent and level of expression. 30-60% of mouse bone marrow cells maintained in culture after infection express hADA when analyzed by this technique. Ongoing transplant experiments are evaluating various parameters which may improve infection efficiency of pluripotent stem cells. Additional vectors with tissue specific enhancer and ADA promoter sequences have been developed for study in this model system. These studies will provide insights into long term tissue specific expression following retroviral mediated gene transfer.

H 219 MONOCLONAL ANTIBODIES TO AN IL-3-BINDING PROTEIN ON MURINE MAST CELLS. P.A. Morel, K. Townsend, M. Gross, D.J. Tweardy and J. Chiller. Pittsburgh Cancer Institute, Pittsburgh, PA 15213 and Eli Lilly Research Facility, La Jolla, CA 92037.
Interleukin-3 (IL-3, multi-CSF) has numerous functions in hematopoiesis, yet its receptor has not been fully characterized. We have developed monoclonal antibodies that interfere with the function of IL-3. Fisher rats were immunized with a bone marrow derived mast cell line that is IL-3 dependent for growth. Antibodies are selected that could inhibit IL-3-dependent but not IL-2-dependent growth. Two monoclonals with these properties, 4G8 and 2F2, were selected for further study. Immunofluorescence staining patterns using 4G8 and 2F2 revealed that these antibodies brightly stained all IL-3 dependent lines tested, normal T cell clones and T cell hybridomas, Wehi-3 and P815. They did not stain B cells and BW5147. In normal tissues, 4G8 and 2F2 stained the myeloid cells of the bone marrow, all thymocytes, 60% of lymph node cells and 30% of spleen cells. Western blot analysis demonstrated that these antibodies recognized a cell surface protein of 110 kD. Cross-linking experiments using iodinated purified IL-3 revealed two labelled bands at 60 kD and 85 kD (after subtracting the contribution of IL-3). Binding of IL-3 to both of these molecules was inhibited by preincubation with 4G8 and 2F2 but not with R17 (anti-transferin receptor). Receptor binding studies revealed that 4G8 was able to inhibit binding of IL-3 to the cell lines, MC9 and FDPC.2, whereas 2F2 was not. However, 4G8 was also able to inhibit IL-4 binding to MC9 cells. These results suggest that 4G8 and 2F2 recognize different epitopes of a molecule present at moderate to high density on several cell types; this molecule either binds IL-3 itself or is very closely associated with the high affinity IL-3 receptor.

H 220 PURIFICATION AND CHARACTERIZATION OF THE INTERLEUKIN-3 RECEPTOR, Alice L-F. Mui, Poul H.B. Sorensen, Robert J. Kay and Gerald Krystal. Terry Fox Laboratory, University of British Columbia, Vancouver, B.C. Canada V5Z 1L3. Murine interleukin-3 (IL-3) is a hemopoietic growth factor that stimulates the proliferation and differentiation of both committed progenitors and pluripotent stem cells. However, little is known to date about the mechanism of action of IL-3 other than it increases intracellular levels of ATP and glucose, activates protein kinase C and induces the serine and tyrosine specific phosphorylation of a select group of proteins. Integral to our further understanding of how IL-3 stimulates its target cells is the purification and characterization of its cell surface receptor. In pursuit of this goal, two assays capable of detecting membrane free, detergent solubilized IL-3 binding activity have been developed and used to follow the IL-3 receptor during purification. Our purification protocol begins with an affinity chromatography step involving biotinylated IL-3, purified plasma membranes from B6SUtA$_1$ cells and streptavidin-agarose. This step yields a receptor preparation which is 150 fold enriched. Further purification, utilizing DEAE-Sephacel and wheat germ agglutinin sepharose columns, suggests that the IL-3 receptor is a complex of proteins, the most apparent being a 140 kd glycoprotein. Studies involving ^{32}P-labelled cells, biotinylated IL-3 and streptavidin-agarose indicate that this 140 kd protein is rapidly phosphorylated on tyrosine residues in response to IL-3. Moreover, 5-p-fluorosulfonylbenozyl [8-^{14}C] adenosine labelling experiments suggest that this 140 kd component of the IL-3 receptor might possess an intrinsic tyrosine kinase activity. This protein is presently being isolated in quantities sufficient for sequence analysis in order to resolve this issue and to allow the cloning of its gene.

H 221 EXPRESSION AND REGULATION OF GM-CSF RECEPTORS ON THE BLAST CELLS OF ACUTE MYELOBLASTIC LEUKEMIA, N. Onetto, C. Bigras, N. Aumont, S.C. Clark, A. De Lean, T. Hoang, Clinical Research Institute, Montreal, PQ, H2W 1R7 and Genetics Institute, Cambridge, MA 02140
Specific binding of GM-CSF to a high affinity receptor was observed on AML blasts (8 patients); the levels of binding were, however, quite heterogeneous among different patient samples. Two categories of AML blasts have been identified with regard to GM-CSF binding: those displaying relatively high levels of binding (63 to 106 molecules of GM-CSF bound per cell at 135 pM) and those with low levels (5 to 21 molecules). Saturation analyses indicated that heterogeneity in binding can be attributed to differences in the average number of binding sites per cell, rather than variations in the affinity between ligand and receptors. The observed heterogeneity in binding was not due to receptor occupancy by an endogenous source of GM-CSF. In fact, the specific signals of AML cells with either high or low levels of binding were not modified by acid wash, a procedure that can remove more than 80% of surface bound GM-CSF. Preincubation of AML blasts with cold GM-CSF induced a reversible internalisation of GM-CSF receptors. The physiological importance of this receptor internalisation in the generation and/or the control of the cellular response is presently under investigation.

H 222 HEMATOPOIESIS AND GENERATION OF GRANULOCYTE-MACROPHAGE PRECURSORS IN A STROMA-FREE LONG TERM BONE MARROW CULTURE SYSTEM, D.W. Pietryga and M.L.R. Ramnaraine, Div. Hematology / Oncology, Children's Hospital Medical Center, Cincinnati, OH 45229.
Long term bone marrow cultures (LTBMC) have been shown to require a viable stromal cell layer for proliferation of nonadherent (NA) hematopoietic cells and generation of granulocyte-macrophage precursors (CFU-GM). To circumvent the need for the stromal layer, NA cells from mature LTBMC were plated at 2.5 x 10^6/ml with varying amounts of 5637 SN (IL-1, G-CSF, GM-CSF), WEHI-3 SN (IL-3) and horse serum (HS) totaling 20% (v/v) of the culture medium (total volume of 8 ml). Hydrocortisone (10^{-7} M) was added to all cultures which were grown at 33°C in 5% CO_2 and demidepleted weekly for up to 14 weeks. Cultures with only 20% HS lacked NA cells after 2 weeks and developed a stromal layer. Cultures with HS and both SNs (2.5% 5637 SN + 2.5% WEHI-3 SN or 5% 5637 SN + 5% WEHI-3 SN) maintained cell count greater than the initial cell count for the first 2 weeks. A rapid drop to <10% initial cell count followed. Total CFU-GM were likewise high initially, but steadily decreased after week 3. No stroma developed. Cultures with IL-3 (5% WEHI-3 SN or 1% WEHI-3 SN) maintained cells at 40% of initial count for 2 weeks but rapidly declined to <10% of the initial count. A semi-confluent stroma formed. Total CFU-GM production remained high (range = 370.4 ± 14.7 to 4938 ± 845.7) for 10 weeks. Cultures with 10% 5637 SN had rapid loss of NA cell production and loss of CFU-GM in 2 weeks. No stromal cell layer developed. Cultures with 5% 5637 SN had an initial drop in cell count, but a steady increase to >80% of initial count during weeks 5-14. CFU-GM remained high, steadily increasing to >10,000 during weeks 12-14. No stroma developed in these cultures. These studies demonstrate that exogenous growth factors can support the growth of NA bone marrow cells and CFU-GM in the absence of a stromal cell layer in long-term culture. The use of purified growth factors in this culture system should allow the elucidation of the precise requirements for long-term stromal-free growth and development of hematopoietic cells.

H 223 EFFECT OF PLATING TECHNIQUE ON HEMATOPOIESIS IN MURINE LONG-TERM BONE MARRROW CULTURES (LTBMC), M.L.R. Ramnaraine and D.W. Pietryga, Division Hematology / Oncology, Children's Hospital Medical Center, Cincinnati, OH 45229.
Various techniques for establishment of murine LTBMC differ in their ability to initiate and maintain production of nonadherent hematopoietic cells (NA) and colony-forming progenitors (CFU-GM). Cultures were established by: 1) recharge of 10^6 fresh bone marrow cells (BM)/culture onto a pre-established stromal layer, 2) plating a "femur equivalent" of pooled and resuspended BM, 3) plating resuspended BM at varying concentrations (see table). NA cell count, ^{3}H-thymidine uptake and CFU-GM were assessed at weekly demi-depletion. Establishment of stromal layer and rapid decrease in NA were noted during the first 3-4 weeks. Cultures which did not begin to increase NA count at weeks 5-7 never did so and remained at <10^5 NA/culture. Production of NA was directly related to number of cells initially plated (p<0.0001). CFU-GM were similar in all cultures for the first 3 weeks but after 5 weeks were higher in those with the larger inocula (p<0.01). Resuspended BM cells can establish an efficient LTBMC without need for pre-established stroma. Characteristics of cell growth are significantly altered by the size of the inoculum.

Technique	No. Failures (%)	NA (# x 10^6/ml)*	CFU-GM (#/10^5cells) *	
1. Recharge	1/7 (14%)	0.40 ± 0.63 (54)	nd.†	* mean ±SD (N); Σwks. 5-12
2. Femur Equivalent¶	0/5 (0%)	0.49 ± 0.27 (25)	nd.†	
3. a) 20 x 10^6/flask	0/5 (0%)	0.86 ± 0.45 (30)	27.6 ± 8.0 (20)	† not determined
b) 15 x 10^6/flask	0/5 (0%)	0.98 ± 0.32 (30)	31.5 ± 11.2 (20)	
c) 10 x 10^6/flask	1/5 (20%)	0.44 ± 0.31 (30)	21.3 ± 12.9 (20)	¶ 2 x 25 cm^2 flasks per mouse
d) 5 x 10^6/flask	4/5 (80%)	0.05 ± 0.12 (30)	13.8 ± 3.9 (5)	

all cultures in αMEM, 20% horse serum, 100U/ml pen, 100µg/ml strep and 10^{-6}M hydrocortisone at 33°C, 5% CO_2

H 224 CHARACTERIZATION OF THE HUMAN GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR RECEPTOR FROM PLACENTA,
Julie E. Scheffler, Lorraine C. Fleissner, Gail F. Seelig, Tattanahalli L. Nagabhushan, and Paul P. Trotta, Dept. of Biotechnology-Biochemistry, Schering Corp. Bloomfield, N.J. 07003
The receptor for granulocyte-macrophage colony-stimulating factor (GM-CSF) has been characterized on particulate membranes derived from human placenta. ^{125}I-GM-CSF binding was specific and saturable, exhibiting a Kd of 0.7 nM (receptor concentration = 95 ng/mg protein). The affinity of ^{125}I-GM-CSF for the placental membrane receptor was similar to that observed for binding to choriocarcinoma JAR cells in culture (Kd = 2-4 nM; 40,000-60,000 receptors/cell). In contrast, the affinity of ^{125}I-GM-CSF for the receptor on KG-1 and AML-193 cells was significantly higher (Kd = 5-10 pM). The molecular weight estimated by covalent cross-linking of ^{125}I-GM-CSF to placental membranes followed by gel electrophoresis and autoradiography was 83,000 which is identical to the molecular weight observed for the receptor on KG-1 cells. A neutralizing monoclonal anti-GM-CSF antibody that blocks binding of ^{125}I-GM-CSF to KG-1 cell receptors also blocks binding to the placental receptor. These results suggest that the high and low affinity GM-CSF receptors on human cells are structurally similar.

H 225 BIOCHEMICAL CHARACTERIZATION AND PARTIAL PURIFICATION OF THE MURINE INTERLEUKIN 3 RECEPTOR, Jolanda Schreurs, Ken-ichi Arai, and Atsushi Miyajima, Molecular Biology Department, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304. We have previously shown that: the IL-3 receptor has both a high and low affinity binding component; IL-3 stimulates tyrosine phosphorylation; and multiple membrane proteins (M_r= 140, 115, 90, 75, 65 kD) can be cross-linked by IL-3. To definitively characterize the IL-3 receptor will require its purification. Thus, we have developed binding assays for membrane and detergent solubilized preparations of the IL-3 receptor. Binding of IL-3 to the receptor is influenced by pH, salt, and divalent cations. Optimal binding conditions for membrane preparations of the receptor display a biphasic pattern of binding, in which the high affinity component has a dissociation constant of 5nM. Detergent solubilized receptor retains its binding capacity in a variety of detergents including Triton X-100, CHAPS, and octylglucoside. IL-3 stimulated tyrosine kinase activity was not detectable in intact or detergent-solubilized membrane preparations, in contrast to the marked tyrosine phosphorylation found with intact cells. Using glycosylated silk-worm derived IL-3, we have developed purification procedures for the solubilized receptor using IL-3 affinity chromatography and ligand blotting.

H 226 DIFFERENTIAL EXPRESSION OF INTERLEUKIN-6 (IL-6) RECEPTORS ON HUMAN MONOCYTIC-, NK-, AND B-CELL LINES, Michael Schwabe[1], Fabrizio DeBenedetti[1], Steve C. Clark[2] and Connie R. Faltynek[3], Laboratory of Biochemical Physiology-BRMP[1] and BCDP-Program Resources, Inc.[3], NCI-FCRF, Frederick, MD, and Genetics Institute[2], Cambridge, MA.
We wanted to determine whether the diverse biological effects of IL-6 are mediated by the interaction of this cytokine with a single class of binding sites on different target tissues. Performing affinity-crosslinking experiments with ^{125}I-labeled IL-6 revealed a similar expression of IL-6 receptors on HL-60, U-937 and THP-1 cells with a major complex in the range of 135 kD and two minor bands of 145 and 160 kD. This crosslinking pattern was slightly different on YT cells with a major receptor complex of 115 kD. On SKW 6.4 cells we detected a clearly distinct pattern of three equally prominant receptor complexes of 160, 150 and 140 kD. However, Scatchard analysis on these cell lines revealed a single class of binding sites with affinity constants ranging from 1 x 10^{-10}-7 x 10^{-10} M. The number of binding sites/cell ranged between 3000 (SKW 6.4) and 10 000 (U-937,THP-1). We conclude that the IL-6 receptor is differentially expressed on hematopoietic cell lines and that apparent size differences in receptor molecules are not necessarily reflected by marked changes of their affinity constants. We postulate that tissue specific modifications of the IL-6 receptor may be of biological significance in the mode of action of this cytokine.

H 227 Mo1 (C3bi) RECEPTOR EXPRESSION AND PMN ADHERENCE ARE ENHANCED BY RhGM-CSF IN THE NEONATAL PMN, L. Sender, C. VandeVen, Y. Suen, and M.S. Cairo, Childrens Hospital of Orange Co., Childrens Hospital of Los Angeles, U.C. Irvine; Orange, CA 92668. Neonatal PMNs have been demonstrated to have reduced expression of the adherence protein, Mo-1, (Bruce et al Ped. Res. 21:306, 1987) and diminished ability to respond to a variety of physiological stimuli. This study investigated the priming effect and direct stimulatory effect of rhGM-CSF (0-2000 pMol/L) on neonatal (cord) PMN expression of Mo-1 (C3bi) and (0-200 pMol/L) PMN adherence. Cord PMNs were isolated by Ficoll-Hypaque density centrifugation and incubated with rhGM-CSF (Amgen, 4 x10^7 unit/mg) for 0-15': Mo-1 surface receptor expression was quantitated by indirect-immunoflourescence using the CD11b (Becton-Dickenson) Monoclonal Ab and FACS analysis. RhGM-CSF dramatically induced Mo-1 expression at 15': (250;500;1000 pMol/L) 129.4 $\pm$ 5.3% of C, ($p < .001$) 141.5 $\pm$ 4.1% of C ($p < .001$) and 150.2 $\pm$ 5.3% of C, ($p < .0001$) respectively. Cord PMNs were incubated with rhGM-CSF (1000 pMol/L) (5') and then stimulated with A23187. This also resulted in significant expression of Mo-1 122 $\pm$ 4.5% of C ($p < .001$). PMN adherence was then measured by % nylon wool adherence after rhGM-CSF incubation (10') (50 & 100 pMol/L) (117.9 $\pm$ 8.3% of C and 131 $\pm$ 5.7% of C ($p < .04$). RhGM-CSF (100 pMol/L) also primed NB PMNs for adherence prior to A23187 stimulation 107.9 $\pm$ 0.7% of C ($p < .02$). This preliminary data suggests that rhGM-CSF induces upregulation of the surface receptor Mo-1 in N.B. PMNs and promotes adherence with and without another physiological stimulus. Further studies are underway to define the clinical relevance of these cellular phenomena.

H 228 INDUCTION OF DIFFERENTIATION BY EGF ON AN EGF RECEPTOR-EXPRESSING IL-3 DEPENDENT CELL LINE, Huey-Mei Wang, Ken-ichi Arai and Atsushi Miyajima, Department of Molecular Biology, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304. Interleukin 3 (IL-3) is a T-cell derived lymphokine which regulates the proliferation and differentiation of hemopoietic cells. It has been demonstrated that IL-3 stimulates tyrosine phosphorylation of several proteins including a membrane glycoprotein and some soluble proteins. Along the same line, IC2 cells infected with Abelson virus carrying the temperature sensitive tyrosine kinase required IL-3 only at high temperature, indicating that tyrosine phosphorylation is necessary for the abrogation of IL-3 dependence. In order to see if tyrosine phosphorylation is essential for IL-3 stimulation, we introduced the EGF receptor gene into an IL-3 dependent pre-mast cell line, IC2, by using retroviral infection. The EGF receptors expressed in infected cell clones exhibit both high and low binding affinities. EGF can transiently substitute for the requirement for IL-3. Furthermore, IL-3 and EGF together act on the infected cells to induce maturation. Detailed biochemical analysis of signal transduction in IC2 and infected IC2 cells is under investigation.

H 229 IMPROVED EFFICIENCY OF RETROVIRAL MEDIATED GENE TRANSFER AND EXPRESSION IN PRIMATE HEMATOPOIETIC PROGENITORS FOLLOWING 5-FU-INDUCED BONE MARROW SUPPRESSION AND RECOVERY, Robert Wieder[1], Ken Cornetta[1], Steven Kessler[2] and W. French Anderson[1], [1]NHLBI, NIH, Bethesda, MD 20892 and [2]Naval Medical Research Institute, Bethesda, MD 20814
Retroviral mediated gene transfer has been carried out into hematopoietic progenitors following 5-fluorouracil (5-FU)-induced bone marrow suppression in mice. In primates, the infection of progenitors by murine retroviral vectors has been less efficient. To define conditions for improved infection rates, we studied the kinetics of 5-FU-induced bone marrow suppression and recovery in 3 rhesus monkeys. Serial bone marrow samplings on days 0, 3, 7, 15 and 24 following a 200 mg/kg IV bolus of 5-FU revealed a decrease in marrow cellularity, in the percent CD34+ cells and in CFU-C on days 3 and 7 and a recovery on day 15. The percent of cells not in G1/Go were 15, 7, 8, 11 and 14 on these days. The percent G418 resistant CFU-C following N2 supernatant infections were 6, 1.5, 0, 30 and 3 on the same days. Increased infection rates of progenitors correlated with increased cycling of the progenitor-enriched populations as shown by albumin density gradient separation of mononuclear cells. In vivo cycling with 5-FU can be applied in autologous bone marrow transplant/gene transfer experiments to optimize retroviral infection of progenitors.

H 230 REGULATION AND SECRETION OF PLASMINOGEN ACTIVATORS AND THEIR INHIBITORS IN A HUMAN LEUKEMIC CELL LINE (K562), E.Lynette Wilson and Lisa Oliver, Dept. of Cell Biology, NYU Medical Center, 550 First Ave., New York, NY 10016.
We have shown that the species of plasminogen activator (PA) secreted by normal bone marrow stem cells is a differentiation linked event with the most primitive progenitor cells secreting tissue PA (t-PA) and more mature cells secreting urokinase (u-PA). In order to analyse the shift in PA synthesis from t-PA to u-PA during differentiation we have characterized the regulation of these enzymes and their inhibitors in K562 cells. K562 cells normally secrete both t-PA and u-PA in a ratio of 3:1. Following the addition of 10 or 1 ng/ml phorbol myristate acetate (PMA) to K562 cells, a marked decrease in enzymatic activity is observed in the medium. However, when t-PA antigen rather than activity is measured, an increased amount is found in the medium. PMA also induces the secretion of the two inhibitors of PA: PA inhibitor 1 (PAI-1) and PA inhibitor 2 (PAI-2). This accounts for the decrease in total enzymatic activity under conditions when the production of t-PA antigen is increased. A study of the time course of induction revealed that the synthesis of PA occured before that of its inhibitors. Low concentrations of PMA (0.1 ng/ml) induce primarily t-PA antigen and not the inhibitors. This results in an increase in total enzymatic activity with 94% of the secreted activity being t-PA. Thus, the secretion of PA and its inhibitors can be manipulated in certain leukemic cells by inducers such as PMA. Secretion of these proteases and their inhibitors by normal marrow or leukemic cells may affect the generation of local haemopoietic growth factors and may be relevant in normal stem cell and leukemic cell/stromal cell interactions.

Gene Expression; Stem Cells

H 300 MAPPING OF THE hIL-4 REGULATORY REGION THAT MEDIATES THE RESPONSE TO HTLVI P40^{tax}, BPV E2 AND PMA/A23187, Etsuko Abe, Mitsuaki Yoshida*, Ken-ichi Arai, and Naoko Arai, Department of Molecular Biology, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304; *Cancer Institute, Tokyo, Japan. We previously reported complete nucleotide sequence of the human IL-4 gene which is composed of four exons and three introns. This gene is approximately 10 Kb in size and is mapped on chromosome 5[(1)]. The IL-4 gene is activated after treatment of human T cell lines by phorbol ester (PMA) and calcium ionophore (A23187). Transient transfection experiments showed that 5'-flanking region is responsible for activation of the hIL-4 gene. In this region, octamer enhancer motif sequence (ATGCAAAG) of SV40 72 bp repeat and conserved lymphokine element (CLE[(2)]; AAGGTTTCAT) which has 60% homology with mGM-CSF are found at positions -276 to -269 and -169 to -160 from TATA like sequence, respectively. In order to study the activation mechanism of the IL-4 gene, we generated a series of deletion derivatives having various length of 5'-flanking sequences. Since hIL-4 promoter is not strong, hIL-4 having various length of 5'-flanking sequences was fused with mGM-CSF promoter (at position -60) lacking regulatory elements and was characterized. Fusion promoter was weakly activated by E2, P40^{tax} or PMA/A23187 stimulation. However, combination of transactivator and PMA/A23187 gave synergistic effect on transcription activity. The region responsive to those stimulations was mapped within 175 bp upstream from transcription initiation site.

(1) N. Arai et al. (1988) J. Immunology, in press.
(2) S. Miyatake et al. (1988) Nucl. Acid Res. 16, 6547.

H 301 Partial purification of T-stem cells and their requirement for growth

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Our previous study and others have shown that functional T cell repertoire can generate in thymic milieu from a single stem cell for T cell-lineage(T-stem). In the present report, We investigated to purify T-stem cells from bone marrow, and the requirement for their growth were examined. Bone marrow cells from B10Thy1.1 mice were prepared,and incubated on M1/70 coated dishes. Then, non-adherent cell were recovered, and fractionated by centrifugation on a discontinuous BSA gradient. Low density cells (less than 1.070) were collected. After washing these cells were further treated with anti-Ia, anti-Thy1.1, Antileukocyte(NEI031) J11d and C. T-stem cell activities of these cells and control bone marrow cells were examined by the intrathymic injection of various number of cells into irradiatd C57Bl/6 mice. T-stem cell frequency was increased about 20 fold by these treatments as compared with non-treated bone marrow cells which have T-stem cell frequency about 1/10000. We will discuss the growth requirement of T-stem enriched populatiuon for the maintenace of T-stem cell activity in vitro.

H 302 INDUCTION OF THE EXPRESSION OF PROTO-ONCOGENES c-fms, c-myc AND c-fos DURING MONOCYTIC DIFFERENTIATION BY A PHORBOL ESTER TUMOR PROMOTER IN A HUMAN MONOCYTIC LEUKEMIA CELL LINE THP-1. Nelleke Barendsen, Mark Mueller and Ben Chen, Div. Hematology-Oncology, Department of Internal Medicine, Wayne State University, Detroit, MI 48201. The relationship of the proto-oncogenes c-fms, c-myc and c-fos expression to 12-O-tetradecanoylphorbol-13-acetate (TPA) induced monocytic differentiation in a human acute monocytic leukemia cell line, THP-1, was investigated. After TPA treatment, the THP-1 cells became adherent with marked morphologic changes, lost division potential, acquired Fc receptors and expressed enhanced functional activities. Treatment with DMSO, rHuM-CSF, rHuGM-CSF or rHuIL-2 failed to induce monocytic differentiation of THP-1 cells. Northern blots were probed with a 1.6-kb ClaI/EcoRI fragment of the human c-myc exon 3, the 2.3-kb XhoI/NcoI fragment of the c-fos gene and the 0.9-kb EcoRI/HindIII fragment containing part of the 5th exon of the human c-fms oncogene. The expression of both c-fms and c-fos transcripts were induced within 1 hr following TPA induction and declined thereafter to nearly undetectable levels after 24 hrs. A rapid and transient down-regulation of c-myc RNA transcripts was observed following TPA treatment which returned to normal level within 24 hr. Prolonged treatment with TPA thereafter resulted in a steady decline of c-myc expression in THP-1 cells concomitant with a decreasing proportion of proliferating cells. Despite the induction of c-fms transcripts, however, no CSF-1 receptors were detected on the THP-1 cell surface after TPA treatment. These findings suggest that c-fms and c-fos may play a regulatory role in the terminal differentiation of cells of the monocytic lineage.

H 303 **L3T4-POSITIVE HEMATOPOIETIC PRECURSORS.** Ross S. Basch and Gay Fredrickson, Dept. of Pathology, N.Y.U Medical Center, New York, NY 10016

A subset of peripheral T cells express a surface molecule (CD4) which is believed to interact with Class II MHC antigens. The murine equivalent, designated L3T4, has a m.w. of 52,000 and is expressed by approximately 90% of thymocytes and 20% of spleen cells. Functional, biochemical and flow cytometric data indicate that L3T4 is expressed by the helper/inducer subset of murine T-cells and is similar to the human Leu3/T4 molecule. A physical interaction between CD4+ T cells and Ia+ bearing cells has been demonstrated which is strong enough for cell-cell binding to occur in the absence of other adhesion factors.

L3T4 is not however restricted to thymocytes and mature T-cells. Many (20-60%) mouse bone marrow cells express the L3T4 antigen. These cells are immature hematopoietic cells. Sorting bone marrow cells on the basis of their expression of this antigen produces populations of cells that are markedly enriched for multipotential stem cells (CFU-s) and for myeloid precursors (CFU-c). We believe that L3T4 is transiently expressed by most, if not all hematopoietic precursors early in their maturation. These results suggest that L3T4, presumably through its capacity to interact with Class II MHC molecules, plays a role in cell interactions far beyond that already demonstrated in the immune system. We suggest that the expression of CD4 molecules on the surface of immature precursors is required for their interaction with cells within the Hemopoietic Inductive Microenvironment(s) (HIM) of the marrow and thymus.

H 304 **INTERLEUKIN 3 (IL3) IS TRANSCRIPTIONALLY ACTIVATED IN MURINE T CELLS.** **Matthias Bickel and Dov H. Pluznik.** Division of Cytokine Biology, Center for Biologics Evaluation and Research, FDA, Bldg 29A , HFB-800, Rm 2B20, NIH, Bethesda MD, 20892.

The colony stimulating factors (CSF) IL3 and granulocyte/macrophage CSF (GM-CSF) are simultaneously expressed in murine T cells. Biologic activities of IL3 and GM-CSF are induced and released into the cell culture supernatant.We have previously shown that by treating the cells with cyclosporin A (CsA) the synthetic pathways of these CSFs are dissociable in T cells. CsA inhibited IL3 but not GM-CSF activity. Since CsA is thought to act at the transcriptional level and only on inducible genes expressed by T cells, we explored in the present study whether the IL3 gene is under a transcriptional control mechanism. In preliminary experiments, we tested whether *de novo* synthesis of RNA is required for the expression of the biologically active protein. The T cell line EL-4 was treated with an inhibitor of transcription initiation, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) after stimulation with a phorbol ester (TPA). In contrast to what we observed with GM-CSF, IL3 was completely inhibited by this inhibitor, similar to what has been reported for IL2. In additional experiments, we compared the steady-state levels of the IL3 and GM-CSF mRNAs utilizing an in-solution hybridization protocol, followed by chromatographic separation of double-stranded from single-stranded RNA. Results of these experiments showed the appearance of IL3 mRNA in TPA-stimulated cells only, while GM-CSF transcripts were detected both in unstimulated and TPA-stimulated cells. To directly demonstrate a transcriptional activation of the IL3 gene, a nuclear transcription analysis was performed. Comparing nuclear runoff transcription in unstimulated and TPA-stimulated EL-4 cells revealed that the IL3 gene is silent in unstimulated but transcriptionally active in stimulated cells, whereas the GM-CSF gene was transcriptionally active both in unstimulated and TPA-stimulated cells. These findings indicate that the control of the IL3 gene is similar to that of IL2, and is distinct from the control mechanism of the GM-CSF gene.

H 305 CONSTITUTIVE EXPRESSION OF THE IL-6 GENE IN CHRONIC LYMPHOCYTIC LEUKEMIA, *Biondi A.,*Rossi V.,°Bassan R.,°Barbui T.,°°Bettoni S.,"Sironi M.,"Mantovani A. and °°Rambaldi A.,*Clinica Pediatrica Univ.di Milano-Osp.S.Gerardo,Monza,°Div.Ematologia Osp.Riuniti di Bergamo,°°Lab.Immunologia Umana e "Laboratori "Negri Bergamo",Ist.Ricerche Farmacologiche "Mario Negri",Milano.

IL-6 is a pleiotropic lymphokine active as a growth factor on B cell hybridomas and plasmocytomas and found to be identical with B cell stimulatory factor 2,Interferon beta-2,26-Kd-protein and hepatocytes stimulating factor. IL-6 gene expression was investigated in fresh human Chronic Lymphocytic Leukemia (B-CLL) and Acute Lymphoblastic Leukemia (ALL) by Northern blot analysis using a specific cDNA probe. 1.3 Kb IL-6 transcript was found in 6 out of 11 B-CLL patients while no hybridization was observed in 10 cases of ALL of both T and B cell origin. The constitutive expression of IL-6 transcripts was associated with production of a biologically active protein as determined by using the IL-6 dependent 7TD1 cell line. It remains to be elucidated whether IL-6 gene expression is indeed important in the regulation of B-CLL growth or in its clinical manifestation.

H 306 DIFFERENTIATION OF DENDRITIC CELLS FROM RAT BONE MARROW CELLS, William E. Bowers, Mary S. Ruhoff and Estelle M. Goodell, Medical Research Institute, Bassett Hospital, Cooperstown, NY 13326

Rat dendritic cells (DC) function as potent stimulators of a mixed leukocyte reaction and as the accessory cell required for lymphocyte responses to the mitogen, sodium periodate. DC have been purified from lymph node cell suspensions and are non-adherent, of low density, Ia^+, FcR^-, and unaffected by γ-irradiation in their ability to function as stimulator or accessory cells. In contrast to preparations of lymph node cells, those of bone marrow (BMC) have extremely low levels of accessory activity and lack recognizable DC. However, after culture of BMC for 4-5 days, DC appear that are functionally and morphologically indistinguishable from lymph node DC. More than 90% of the DC originate from a low density fraction (LD-BMC) that contains 5% of the total BMC. γ-irradiation of LD-BMC prior to culture prevents the development of DC and accessory activity. For individual cultures of LD-BMC irradiated on successive days, both the number of dendritic cells and the total accessory activity increase daily to reach levels 20-30-fold higher than those on day one. Removal of Ia^+ cells from LD-BMC prior to culture has little effect on the number of DC or the total accessory activity found at 5 days. Both lymph node DC and DC from 5-day cultures of LD-BMC can be removed by the same panning procedure. Rosetting and fluorescence-activated cell sorting, in conjunction with a panel of monoclonal antibodies, including some we have produced against dendritic cells, are being used to identify the phenotype of the dendritic cell precursors. The results suggest that in bone marrow, DC develop through division of precursors that have alow density and lack Ia.

H 307 A NEW APPROACH TO ANALYZING THE MODULATION OF DNA-BINDING PROTEINS INVOLVED IN T CELL ACTIVATION, Anna T. Brini, Annick Harel-Bellan, Marie Korner and William L. Farrar. Laboratory of Molecular Immunoregulation, NCI-FCRF, Frederick, MD 21701-1013. We have utilized a rapid and convenient method to detect and analyze interactions between cis-regulatory elements and transactivating factors in T cells. Radiolabeled double stranded oligonucleotides corresponding to the HIV enhancer and to the IL 2 receptor alpha chain enhancer sequences were electroporated into Jurkat cells or into purified resting human T cells. After different treatments and various periods of culture the cells were washed, lysed and the cell lysates were analyzed by non-denaturating polyacrylamide gel electrophoresis and autoradiography. Here we show that the proteins that bind to these enhancer sequences are specific since electroporation of an excess of cold HIV or IL 2 receptor alpha chain sequences inhibit the DNA proteins complexes, while unrelated sequence (CRE) does not. Furthermore, T cell activation by PHA or PHA-PMA increases the complex formation which was not affected by cycloeximide and actinomycin D. These results suggest that these DNA-binding proteins pre-exist in the cell and that mitogens could activate specific DNA-binding proteins by posttranslational mechanisms or by acting on an inhibitor. Since these two enhancer sequences present 80% sequence identity and the DNA-protein complexes behave similarly we suggest that some of the proteins which recognize them are identical.

H 308 ***IN VITRO*** **TRANSCRIPTION OF THE HUMAN ERYTHROPOIETIN GENE.**
Costa- Giomi, P; Ramírez,S*; Badiavas, E*; Caro,J* and Weinmann, R . Wistar Institute. 3601 Spruce. Philadelphia, Pa. 19104.*Cardeza Foundation. 1015 Walnut St. Philadelphia. Pa 19107.

The Hep3B cell line has been reported to be induced 18 fold to produce erythropoietin (Epo) in response to hypoxia and cobalt (II) chloride (Goldberg et al, 1987). We decided to use the Hep3B cell line as a system to study the regulation of the expression of the Epo gene. Nuclear run-on assays have shown that the induction of the Epo gene in Hep3B is transcriptionally regulated. Nuclear extracts prepared from induced and uninduced Hep3B cells actively transcribe *in vitro* the Epo gene. Primer extension analysis indicated that the initiation site used *in vitro* is identical to the *in vivo* initiation site. Nuclear extracts were used to transcribe *in vitro* deletion mutants of the upstream sequences. In mutants lacking the sequences between -234 and -47 the level of *in vitro* transcription is significantly reduced.

So far we have not been able to reproduce in the *in vitro* system the induction or tissue specificity of the Epo gene. One explanation is that the template used for *in vitro* transcription does not contain all the cis-acting sequences needed for appropriate regulation. Alternatively the activities of some trans-acting factors may be lost in the *in vitro* system. Work is in progress to investigate these possibilities.

H 309 **IN VIVO AND IN VITRO TRANSPLANTATION OF PERIPHERAL BLOOD DERIVED STEM CELLS IN THE MOUSE.** David A. Crouse, Greg A. Perry, Randall Schmidt, Linda Krauter and J. Graham Sharp. University of Nebraska Medical Center, Omaha, NE 68105.

Interest in transplantation of autologous hematopoietic stem cells (HSC) derived from peripheral blood (PB) has recently been rekindled. Transplants of this type may be useful when the marrow has been compromised by prior radiation exposure or by the presence of metastatic tumor. In these studies we compared PB-HSC collected by centrifugation in a silica gradient to bone marrow (BM) HSC obtained from the same BDF$_1$ donors. We examined in vivo transplantation using syngeneic recipient mice which had received a lethal whole body radiation exposure (10.5 Gy, ^{60}Co) 24 hrs prior to transplantation. The number of cells grafted was adjusted to obtain countable CFUs or to provide approximately equal numbers of HSC in the long term reconstitution studies. Initially, we evaluated CFUs content, cycling fraction and reconstitution of various lymphohematopoietic endpoints between 12 and 30 days post-grafting. Both D8 & D12 PB-derived CFUs were present at a frequency of about 7% of BM-HSC leading to an 8:12 day ratio similar to that of BM (~1). Similar percentages of PB and BM-HSC were killed by in vitro hydroxyurea treatment. Lymphohematopoietic recovery lagged in PB-HSC versus BM grafted mice, but at 30 days only thymic reconstitution and GM-CFC remained significantly lower in the PB-HSC group. We examined in vitro transplantation using syngeneic long term marrow cultures which received 10 Gy prior to the addition of 2x10^5 purified PB mononuclear cells. The data from this experiment showed that PB-HSC could reconstitute the hematopoietic compartment of radiation depleted LTMC, however the low number of PB-HSC available for grafting and surprising radioresistance of some stem cells which survived the depleting radiation dose, complicated the interpretation of this experiment. In summary, our data suggest that while the relative primitiveness of PB-HSC and BM-HSC are not different, the reconstitution of some lymphohematopoietic tissues may differ. (Supported by a Nebraska Department of Health LB506 Grant)

H 310 **SYNTHESIS OF A HUMAN INTERLEUKIN 3 GENE.**

R.L. Cutler, T.C. Atkinson*, M. Smith*, A.J. Hapel and I.G. Young. John Curtin School of Medical Research, Australian National University, Canberra, A.C.T., 2601, Australia. *Dept of Biochemistry and Biotechnology Laboratory, University of British Columbia, Vancouver, B.C., Canada,V6T 1W5.

Interleukin 3 (IL3), a multitrophic hemopoietic regulator produced predominantly by T lymphocytes, stimulates the proliferation and differentiation of at least six different hemopoietic lineages. To produce larger amounts of this regulator than can be prepared from natural sources we have turned to gene synthesis and expression in yeast.

The protein sequence of human IL3, deduced from the DNA sequence (1) was used to design a gene encoding the 133 amino acid mature protein. Features of the synthetic gene included: a) use of yeast preferred codons; b) replacement of the codons for Asn 34 and Asn 89, the potential N-glycosylation sites with Asp codons; c) addition of part of a sequence for yeast α-factor leader peptide to enable the mature protein to be secreted from yeast. Both strands of the 420 b.p. duplex were synthesized and the efficient synthesis of the gene was improved by : a) using long oligonucleotides, ranging from 68 to 99 nucleotides in length; b) chemically phosphorylating the oligonucleotides, except for the two oligonucleotides at the 5' ends of the duplex. The protein produced by expression of this synthetic gene in yeast should be useful for studies of the human IL3 protein and its interaction with the human IL3 receptor.

(1) Yang, Y.C., *et al,* Cell,47, 3-10, 1986.

H 311 TRANSCRIPTIONAL FACTORS WHICH REGULATE HUMAN IL-1/HEMOPOEITIN 1 GENE EXPRESSION. Matthew J. Fenton and Jeffrey L. Kosiba, Departments of Medicine and Biochemistry, University Hospital and Boston University School of Medicine, Boston, MA 02118.

Interleukin 1 (IL-1), now known to be identical to the growth factor hemopoietin 1, is a pluripotent cytokine which can synergize with colony stimulating factors to promote proliferation and differentiation of early hematopoietic progenitor cells. Two unique IL-1 genes (proIL-1α and proIL-1β) have been identified in human and murine cells. We have previously reported that human proIL-1β gene expression in monocytic cells is regulated at the transcriptional level by two distinct stimulus-specific pathways (J. Immunol. [1988] 140: 2267). One stimulus (lipopolysaccharide) can induce the transient expression of IL-1β mRNA in THP-1 human monocytic leukemia cells. Nuclear run-on analysis suggests that activation, and subsequent repression at the transcriptional level accounts for this transient response. An additional regulation at the level of IL-1 mRNA stability was also described. The transcriptional controls may be mediated, at least in part, by the interaction of nuclear regulatory proteins with specific nucleotide sequences within the proIL-1β gene. We have used electrophoretic gel mobility shift assays and methylation-interference DNA footprinting to identify two regions within the human proIL-1β upstream sequence which bind distinct nuclear regulatory proteins. One region binds a factor which is present in extracts prepared from transformed lymphocytic and monocytic cell lines, but exhibits an altered mobility shift in extracts prepared from normal human peripheral blood monocytes. A second region contains both the 'TATA box' sequence and the binding site for another nuclear protein which appears to be both monocyte- and stimulus-specific. The factor. This factor may interact with the transcriptional factor TF-IID to promote stimulus-specific induction of proIL-1β transcription in monocytic cells.

H 312 G-CSF PRODUCTION IN PATIENTS WITH PRIMARY NEUTROPENIC DISORDERS, Meri T. Firpo, Miguel Abboud, Ann Jakubowski, Maryanne Maniatis, Mary Ann Bonilla, Richard O'Reilly, Malcolm A.S. Moore, and Janice L. Gabrilove, Sloan-Kettering Institute, New York, NY 10021.

Congenital and idiopathic neutropenia are diseases characterized by a degree of maturation arrest of neutrophil granulocyte precursors and thus a reduction in the number of mature neutrophils. The numbers of eosinophils and monocytes, however, are normal or increased. The gene for human granulocyte colony stimulating factor (hG-CSF) has been cloned and shown to stimulate proliferation and maturation of neutrophils in vivo and in vitro. Recent clinical trials using rhG-CSF in patients with these neutropenic disorders have demonstrated increases in peripheral blood neutrophil counts. The etiology of the disorders and the mechanism of stimulation of maturation by rhG-CSF are not understood. We prepared genomic DNA from peripheral blood buffy coat cells of neutropenic patients. The structure of the G-CSF gene was analyzed by Southern blotting using a cDNA probe. An abnormal genomic pattern of G-CSF was observed in one of seven patients. We also tested fibroblasts, an inducible source of G-CSF, for the ability to produce G-CSF when stimulated. Bone marrow fibroblasts from five patients and two normal donors were cultured and stimulated with rhIL-1 or rhTNF for 72 hours. Conditioned medium from the fibroblast cultures was tested for G-CSF activity by the NFS-60 proliferation assay. G-CSF activity, which could be inhibited by neutralizing antibody to rhG-CSF, was detected in the conditioned media from all cultures tested. Northern analysis of the fibroblast RNA showed G-CSF expression as well. Fibroblast cultures are currently being prepared from the patient showing altered gene structure.

H 313 EXPRESSION OF THE GM-CSF GENE IN MYELOID LEUKEMIA CELLS, Jean Gabert, Nicolas Maroc, Christine Faÿ, Marc Lopez, Diane Razanajaona, Christine Lavezzi, Marie-Josèphe Pébusque, Françoise Birg, Patrice Mannoni, Department of Molecular Hematology, INSERM U. 119 - IPC-CRACM- 232, Bd Sainte-Marguerite, 13009 Marseille - FRANCE.

We have demonstrated that in most cases of human acute myeloid leukemias (AML), leukemic cells respond to and synthesize GM-CSF. Expression of GM-CSF mRNA was detected by Northern blot : the secretion of the corresponding protein by the leukemic cell clone was confirmed by 2 biological assays, both inhibited by neutralizing monoclonal antibodies (mAb) specific for GM-CSF. The relevance of this autocrine loop to leukemic cell proliferation was demonstrated by the inhibition of the spontaneous in vitro growth by addition of anti-GM-CSF mAbs. As GM-CSF is not expressed in normal myeloïd progenitors, we investigated the potential alterations in the regulation of this gene, either at the transcriptional or the post-transcriptional levels, using human leukemic cells, normal monocyte-macrophage cells, macrophage-derived cell lines and tumor cells known to synthesize GM-CSF.

H 314 ACCESSORY CELL MEDIATED ACTIVITY OF GM-CSF ON VERY IMMATURE HEMOPOIETIC STEM CELLS AND COMMITTED MYELOID PROGENITORS, Eugenio Gallo, Daniele Caracciolo, Dario Ferrero, Alessandro Pileri and Corrado Tarella, Department of Medicine and Experimental Oncology, Hematology Section, University of Torino, Italy.

The in vitro stimulating activity of GM-CSF on whole bone marrow (BM) and on highly enriched progenitors depleted of accessory cells was compared to control cultures seeded with standard CSF source (TPA-3O-1 SN). On whole BM, GM-CSF had a colony stimulating activity comparable to control. However, when BM cells were depleted of phagocytizing cells, GM-CSF activity was greatly reduced (27% of control). A further reduction was observed when GM-CSF was tested on highly enriched populations of early and late CFU-GM, separated by means of DS1-1 MoAb (18% and 6% of colony growth, respectively). We then tested the activity of GM-CSF on very immature stem cells surviving after mafosfamide treatment. Mafosfamide kills all cycling progenitors, sparing immature non cycling multipotent stem cells. Mafosfamide resistant cells were incubated for 7 days in suspension cultures, with and without GM-CSF, and then tested for the CFU-GM agar assay. The generation in culture of myeloid clonogenic cells resulted significantly higher in cultures incubated with GM-CSF. However, when accessory cells were removed from the liquid culture, no colony forming cells could be generated. All together, these results suggest that GM-CSF stimulates both committed myeloid progenitors and their totipotent precursors. However, its action is mainly exerted through cooperation with accessory cells.

H 315 REGULATORY ELEMENTS OF THE HUMAN AND MURINE MYELOPEROXIDASE GENE,
Ingrid Gemperlein, Donatella Venturelli, Neelam Shirsat, Keith R. Johnson, Susan Bittenbender, Sheila Hudson, Andrew Engelhardt and Giovanni Rovera, The Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104.

To define sequences involved in lineage and stage specific regulation of expression of the myeloperoxidase (MPO) gene during granulocytic differentiation we isolated and characterized human and murine cDNA and genomic clones. Comparison of the human and murine genes in the 5' region revealed two Alu sequences in the human genome (369bp-709bp and 1522-1793bp upstream of the first ATG-codon of the cDNA) which are not present in the mouse gene. These regions that are further upstream of the putative TATA box identified by Morishita et al, interrupt sequences that have a high degree of homology with the murine MPO gene and are also present in cDNA clones derived from murine and human cDNA libraries. Primer extension experiments and Northern blot analysis of the human mRNA showed that at least two mRNAs differing in their 5' ends are generated by two different transcription start points and differential splicing. Only one MPO mRNA species is transcribed from the mouse gene.

To test for sequences required for regulated gene expression, various regions of the 5' and 3' end of the gene were linked to the coding region of the neomycin phosphotransferase gene and introduced into the human myeloid leukemia HL60 cells and in murine 32D cell line induced to differentiate into promyelocytes by G-CSF treatment. Sequences spanning up to 2700bp upstream of the ATG-codon although sufficient to express low levels of neomycin phosphotransferase in the transfected cells are not sufficient for differentiation-dependent regulation of the gene. Recombinant constructs containing sequences further upstream of the ATG codon and sequences of the 3' end of the gene are presently being analyzed.

H 316 EARLY AND LATE EFFECTS OF HIGH DOSES OF CYTOSINE ARABINOSIDE WITH OR WITHOUT A CFU-S INHIBITOR ON PLURIPOTENT STEM CELL (CFU-S) PROLIFERATION AND DIFFERENTIATION. Guigon M* ,Izumi-Hisha H and Mary JY. INSERM U250, Institut Gustave Roussy, Villejuif, Faculté de Médecine St Antoine, Paris * and INSERM U263, Université Paris 7, France.

High doses of Cytosine Arabinoside (Ara-C) are now used in the treatment of leukemias and in transplant preparative regimens. It is of clinical interest to know if this phase-S specific drug induce long term damage of the pluripotent stem cell compartment as it has been reported for other cytostatics widely used in oncology. Groups of ten SPF CBA mice received 4 injections of about 1g/kg b.w of Ara-C at 0,7, 24 and 30 hours. This protocol led to the death of about 70% animals within 10 days. The administration at 26 hours of a CFU-S inhibitor (4 ug/mouse) partially purified from fetal calf marrow (Wdzieczak-Bakala et al. Biomedicine &Pharmacother1983) resulted in the survival of more than 60% mice. In the survivors of both groups, experiments were carried out 1 day, 1 and 6 months following the last Ara-C injection. CFU-S numbers were studied as well as the determination of their differentiation by evaluating the proportion of splenic colonies of the different lineages. One day after the last Ara-C injection, the number of CFU-S (day 9 colonies) was drastically reduced in both groups. The determination of CFU-S differentiation was altered with an increase in the proportion of granulocytic and megakaryocytic colonies and a decrease in the proportion of erythroid colonies ($p<0.01$). After 1 month, these values return to normal levels and remained unchanged as compared to age-matched controls. The proliferative capacity of CFU-S (R_S) was assessed by the technique of Hellman et al. 1 and 6 months after treatment. R_S values, which were not modified by the administrationof the CFU-S inhibitor alone, appeared to be normal 1 month after treatment and remained within normal values up to 6 months. Therefore, high doses of Ara-C given alone or with a CFU-S inhibitor do not induce long lasting impairment of the CFU-S compartment, although it provokes an intense cycling of these cells in order to allow the hemopoietic recovery.

* Present address

H 317 NOVEL MYELOID SPECIFIC GENES TO STUDY THE REGULATION OF MYELOPOIESIS, LEUKEMIA AND ITS SUPPRESSION, B. Hoffman-Liebermann, Kenneth Lord and D. Liebermann, Department of Biochemisty and Biophysics, University of Pennsylvania School of Medicine, Phil, PA 19104.

Normal and leukemic murine cells of the myeloid lineage, whose growth and differentiation characteristics are amenable for in vitro studies, are being used to investigate fundamental problems in normal cell growth and differentiation, leukemia and suppression of the leukemic phenotype. We have isolated cDNA clones of two types of genes: (1)primary responder genes, induced in the absence of protein synthesis, (and, therefore, comparable to immediate early viral genes) by either growth factor(s) or differentiation factor(s), and (2)genes turned on during normal myelopoiesis, are myeloid specific and are expressed in mature myeloid cells (granulocytes or macrophages). Thus far, one clone of a primary growth responder and numerous clones of the other types were isolated and subjected to preliminary analysis and expression studies in both normal and leukemic myeloid cells. Clones selected for further study include MyG-1, induced by growth factors in normal bone marrow cells in the absence of protein synthesis and constitutively expressed in leukemic cells; MyD-1,2 &3, induced by differentiation factors in both D+ leukemic and normal myeloid cells in the absence of protein synthesis; and My1,2,3,4,& 5, induced during normal myelopoiesis, expressed in mature cells, where My-5 is expressed only in granulocytes and My-4 is expressed only in macrophages, none are expressed in D- leukemic cells and My-4 is not expressed in D+ leukemic cells following treatment with differentiation factor. Currently detailed expresssion analysis and sequencing of the cDNA clones of the selected genes are underway.

H 318 RETROVIRAL TRANSFER OF THE GM-CSF GENE INTO MURINE IL-3 DEPENDENT STEM CELLS: A MODEL FOR REGULATION OF PROLIFERATION AND DIFFERENTIATION, Ursula Just [1,2], Carol Stocking [1], Wolfram Ostertag [1], Elaine Spooncer [2] and Michael Dexter [2]. [1]Heinrich-Pette-Institut fuer Experimentelle Virologie und Immunologie an der Universitaet Hamburg, Martinistrasse 52, 2000 Hamburg, F.R.G. [2]Paterson Institute for Cancer Research, Manchester, M20 9BX.

Multipotential, non-leukaemic, IL-3 dependent, haemopoietic stem cells were infected with a selectable retroviral vector containing the murine GM-CSF gene. The GM-CSF gene was expressed and GM-CSF was secreted from the infected cells in varying amounts dependent on the type of vector used (low or high expression vector). When the cells were cultured with IL-3 in doses optimal for stimulating growth of the cells, the cells remained mainly blasts with some extent of differentiation into granulocytes and macrophages. Control cell lines, infected with the vector containing only the selectable marker, were composed mainly of an homogenous blast cell population when they were cultured in IL-3 and they died in absence of IL-3. When the IL-3 was removed from the medium, most of the GM-CSF-vector-infected cells showed terminal differentiation into granulocytes and macrophages within 7 days of culture. Clonogenic assays in IL-3 containing soft agar indicated that the whole population had irreversibly lost proliferative capability. 10% of the cells infected with the high expression vector however did not differentiate terminally and acquired factor-independent growth autonomy within 7 days of culture without IL-3. This was not observed in cells infected with the low expression vector, indicating that the amount of factor produced correlates with the progression from autocrine stimulation to autonomous growth.

H 319 A SYNTHETIC PEPTIDE DERIVED FROM A RETROVIRAL P15E SEQUENCE DISPLAYS BIOLOGICAL ACTIVITIES OF P15E AND ALPHA INTERFERON, William Kloetzer, Karen Kabat, Don Wegemer and Robert Naso. The Johnson & Johnson Biotechnology Center, San Diego, CA 92121.

Some clinical signs of retrovirus mediated immunosuppression are directly attributed to the envelope protein p15E. A 17 aa immunosuppressive synthetic peptide (ISP) selected from a highly conserved region of p15E is biologically active when chemically cross-linked to BSA (Science 230:453 1985). We describe biological activities of a 29 aa carrier-free peptide (FISP) on con A stimulated murine splenocytes and retrovirus infected cell lines. FISP levels of 11μM to 22μM maximally suppress while peptide levels greater than 22μM have no effect or stimulate [^{3}H]TdR uptake by mitogen activated splenocytes. Passage of splenocytes through nylon wool effectively removes a FISP refractile population of con A responsive cells. Further T cell enrichment by monoclonal antibody mediated cytolysis shows that CD4$^+$ cells are required for suppression of [^{3}H]TdR uptake by FISP. Consistent with this interpretation are results showing that FISP inhibits T_h cell dependent *in vitro* boosting of antibody production as determined in hemolytic plaque assays. The FISP sequence contains an Arg/His/Lys rich domain of p15E linked to the NH_2-terminus of ISP. Analysis of different hybrid peptides indicates that the polycationic tail probably contributes a nonspecific effect while the ISP contribution to biological activity is sequence specific. The ISP sequence is similar to a region of human alpha interferon reported by others to be important for antiviral activity. Electroblots show that FISP levels over 22μM reversibly inhibit virus release from infected feline and murine cell lines in an interferon-like manner. The availability of a synthetic, biologically active peptide should prove useful in further defining the cellular mechanism by which viral p15E mediates its immunosuppressive effects.

Abstract Withdrawn

H 321 DETECTION OF GM-CSF AND IL-3 GENE EXPRESSION IN CULTURED MACROPHAGES BY IN SITU HYBRIDIZATION USING OLIGONUCLEOTIDE PROBES. Bernhard Kubanek, Ivan N. Rich, Christina Vogt and John Lyons. Dpt. of Transfusion Medicine and Sektion Polymere, University of Ulm, D-7900 Ulm/Donau, F.R.G.
Macrophages are probably the most versatile cells in the body capable of sensing and responding to many external signals in order to maintain homeostasis. We have previously demonstrated that unseparated and unfractionated mouse bone marrows cells cultured on hydrophobic Teflon foils give rise to an almost pure population of functionally and phenotypically resident macrophages after 14 days. These cells release Interleukin-3 (IL-3), (measured by an enzymatic assay using the IL-3-dependent FDC-P2 cell line), a colony stimulating factor (CSF) and erythropoietin (EPO), (measured by colony formation in vitro), in the supernatant. To show that 14 day cultured macrophages were not only releasing these regulators but also expressing the genes, oligonucleotide probes (21-mers) for GM-CSF and IL-3 were prepared, labelled at the 3'-end with biotin and used for in situ hybridization. The signal was detected by streptavidin-gold and observed using reflection-contrast microscopy. Negative controls included control DNA and RNase-treated preparations prior to hybridization. Positive controls included the detection of actin gene expression and IL-3 gene expression in WEHI-3 cells. Both GM-CSF and IL-3 oligonucleotide probes demonstrated hybridization to 14 day cultured macrophages, but not all macrophages demonstrated this function, implying that macrophage subpopulations were responsible.

H 322 IDENTIFICATION OF HUMAN LYMPHOHEMATOPOIETIC STEM CELLS WITHIN THE POOL OF INTRATHYMIC SURFACE CD3- CD4-, CD8-, CYTOPLASMIC CD3ε+T CELL PRECURSORS, Joanne Kurtzberg, Steven M. Denning, Lynn M.Nycum, Kay H. Singer, and Barton F. Haynes, Departments of Pediatrics and Medicine, Duke University Medical Center, Durham, NC 27710. The signals and cellular interactions required for the lineage commitment of hematopoietic stem cells to irreversible T cell differentiation are unknown and are central to an understanding of the early stages of normal T cell maturation. To study these events in humans and to determine if intrathymic T cell precursors are irreversibly committed to the T lineage, we isolated CD3-, CD4-, CD8- (triple negative, TN) T cell precursors from human thymus and determined their differentiative capacity into non-lymphoid lineages. We found that cytoplasmic CD3+, surface CD3-, CD4-, CD8- T cell precursors that (in the presence of T cell conditioned medium + IL-2) differentiate into TCR γδ+ cells, are also capable of differentiation into non-lymphoid (myeloerythroid) hematopoietic cells. Clonal hematopoietic progenitors (CFU-GM, CFU-GEMM, and BFU-E) were quantitated within this TN subset. Timecourse experiments revealed that>90% of cells underwent nonlymphoid differentiation within 4-10 days in culture. Further characterization of these cells revealed that prior to differentiation, the majority of cells were CD7+, approximately 50% were CD34+, and that smaller proportions of cells also expressed CD10. The [CD7+, CD34+] and [CD7+, CD34-] subsets were separated and each found to be capable of giving rise to differentiated cells of the lymphoid and myeloerythroid series. We conclude that the intrathymic TN pool of cells that contain T cell precursors also contains cells that are not irreversibly committed to the T lineage. Further, it appears that the lymphohematopoietic stem cell resides within this CD7+ triple negative thymocyte population.

H 323 L-PLASTIN: A HUMAN LEUKOCYTE PROTEIN INAPPROPRIATELY EXPRESSED IN FIBROSARCOMAS AND OTHER SOLID TUMORS, Ching-Shwun Lin[1], Ruedi H. Aebersold[2], Stephen B. Kent[3], Karabi Ghosh[1] and John Leavitt[1], Institute for Medical Research, 2260 Clove Drive, San Jose, CA 95128[1], Biomedical Research Center, University of British Columbia, Vancouver, Canada 16T-1W5[2], and Division of Biology, California Institute of Technology, Pasadena, CA 91125[3]. The phosphoprotein, *L-plastin*, was originally identified as a transformation-induced protein of human tumor-derived fibroblasts and other cancerous cell lines derived from sarcomas and carcinomas. L-plastin is normally expressed in leukocytes as one of the most abundant cytosolic proteins (68kd, pI 5.3). It is not expressed, however, in normal fibroblasts or cells of other solid tissues. Therefore the expression of L-plastin appears to be related to hematopoietic cell differentiation. We and others have shown that L-plastin is polymorphic in charge among humans and that it is phosphorylated at serine residues in response to mitogens and certain lymphokines. Cloning and characterization of two full-length cDNAs encoding plastin has revealed the existence of two distinct isoforms of plastin, the "L" isoform mentioned above and the "T" isoform which is normally expressed in cells of solid tissues, but not in hematopoietic cells. We have isolated genomic clones for both the L and T isoforms in order to identify the gene regulatory elements and mechanism of activation of L-plastin expression in hematopoietic cells. The L-plastin gene is complex in its structure and organization with many large introns and small exons. Characterizations of the regulatory sequences of the promoter and full structure of this gene are in progress. To study the function of the protein, the L-plastin cDNA was inserted into the *beta*-actin promoter expression vector to allow expression of L-plastin in normal fibroblasts. Transfection of this recombinant plastin gene into rodent fibroblasts led to a radical morphological change in the transfectant cells.

H 324 IDENTIFICATION OF ACETYL-N-SER-ASP-LYS-PRO IN FETAL CALF BONE MARROW : A NEGATIVE REGULATOR OF THE PLURIPOTENT HEMATOPOIETIC STEM CELL, Maryse Lenfant[1], Joanna Wdzieczak-Bakala[2], Eric Guittet[1], Jean-Claude Prome[3], Dominique Sotty[1] and Emilia Frindel[2], [1]ICSN/CNRS, 91198 Gif-sur-Yvette, France, France, [2]INSERM U.250 Institut Gustave-Roussy, 94805 Villejuif, [3]CRBGC/CNRS, 31062 Toulouse, France.
We report here a 5-step purification procedure which led to the isolation from fetal calf bone marrow extract of a tetrapeptide Acetyl-N-Ser-Asp-Lys-Pro (m.w. 487 D) exerting a high inhibitory activity on the entry of the hematopoietic pluripotent stem cells (CFU-S) into cycle. The structure of this molecule was established from amino acid analysis, Fast Atom Bombardment Mass Spectrometry and ^1H Nuclear Magnetic Resonance spectral data. This structure was confirmed by comparison with the corresponding synthetic molecule which present identical physicochemical characteristics and biological properties. Both the natural and the synthetic peptides administered to mice, at a dose of 100 ng/mouse, after one injection of cytosine arabinoside, prevent CFU-S recruitment into DNA synthesis.

H 325 QUANTITATION OF THE BINDING OF HUMAN CD34 POSITIVE MYELOID PROGENITORS TO MARROW STROMA, FIBROBLASTS, AND COMPONENTS OF THE EXTRACELLULAR MATRIX. JL Liesveld, DH Ryan, MC Kempski, JK Brennan and CN Abboud. University of Rochester, Rochester, NY.
Hematopoiesis occurs in juxtaposition to cells of the marrow microenvironment such as stromal cells and fibroblasts. To examine the association of progenitors and the microenvironment, we have quantitated the binding capacity of myeloid progenitors to passaged marrow stromal cells, passaged marrow fibroblasts, Triton-X100 extracted matrix (ECM), fibronectin (Fn), laminin, gelatin and plastic. Highly enriched CD34+ cells were obtained from marrow light density (LDM) cells by a two-step flow cytometric procedure. Cells were allowed to bind at 37°C for two hours and the Day 14 CFU-GM growth in the presence of GCT-CM + Mo-CM was used to quantitate the binding of these progenitors. CD34+ cell binding was compared to that of presorted LDM cells. After normalization of the progenitor binding data to that obtained with stromal cells (100%), we found that fibroblasts were 76 ± 21 (n=6 LDM) and 112 ± 15 (n=11 CD34+); Fn was 56 ± 23 (n=7 LDM) and 70 ± 15 (n=10 CD34+); laminin was 80 ± 25 (n=5 LDM) and 119 ± 23 (n=5 CD34+); and plastic was 14 ± 11 (n=7 LDM) and 12 ± 5 (n=7 CD34+). Binding of CFU-GM to extracellular matrix was not significantly blocked by antibody to fibronectin. Also, the presence of RGDS-containing peptides failed to inhibit the CD34+ CFU-GM binding to Triton-X100 extracted matrix, laminin, or fibronectin. These data indicate that myeloid progenitors bind to sites found on marrow stromal cells, fibroblasts, and components of the extracellular matrix. This system can be used to further investigate the specificity of these adhesive sites for progenitor cell subsets which may play a role in the homing and lodgement of stem cells following marrow reinfusion.

H 326 GROWTH AND DIFFERENTIATION OF HEMATOPOIETIC PROGENITOR CELLS
L. London and J.P. McKearn. Searle R&D, Monsanto Co. St. Louis, MO.
The regulation of hematopoietic cellular growth and differentiation involves a complex set of interactions between stem cells, their progenitors and their micorenvironment. We have shown (JEM 166:1419) that CSF-dependent bone marrow cells (FDC-P1) can reside in G_0 and IL-3 is the only factor found to promote a $G_0 \rightarrow G_1$ transition. This $G_0 \rightarrow G_1$ transition is obligatory for the action of other lymphokines/cytokines which support a $G_1 \rightarrow M$ transition. The significance of these finding to normal pluripotent stem cell development is evident since most multipotent stem cells *in vivo* reside in G_0. Unlike IL-4, only IL-3 and G/M-CSF could support clonal growth of FDC-P1 cells. Since FDC-P1 cells possess functionally competent IL-4 receptors, IL-4 may regulate the growth of these cells. IL-4 antagonized the growth of FDC-P1 cells and normal bone marrow cells, in response to IL-3 or G/M-CSF, although growth of bone marrow cells in response to G-CSF or M-CSF was enhanced. Our results suggest that 1) the dual (inhibitory vs. enhancing) role of IL-4 is a direct effect of IL-4 on the CFU target cell and not mediated through accessary cell interactions; 2) one active site on the IL-4 molecule is responsible for mediating its dual effects; 3) IL-4 regulates the functional activity of other CSF receptors; 4) the effect of IL-4 on progenitor cells suggests temporal importance of interleukin action and differentiation status of the target cells.

H 328 CAPACITY OF HUMAN BONE MARROW CELLS TAKEN FOR AUTOLOGOUS MARROW TRANSPLANTATION TO GENERATE ACTIVE HEMOPOIESIS IN MICRO LONG-TERM BONE MARROW CULTURES, H.-G.Mergenthaler[1,2], H.-J.Kolb[2,3], G.Ledderose[2], E.Holler[2], W.Wilmanns[2,3], P.Dörmer[1], GSF-Inst.f.Exp.Haematologie (1), Medizin. Klinik III, Univ.klinikum Großhadern (2), GSF-Inst.f.Klin.Haematologie (3), München, FRG. Patients undergoing autologous bone marrow transplantation usually are heavily pretreated with combination chemotherapy. It is of interest that the period to achieve hemopoietic reconstitution is prolonged in autografted patients when compared to patients allotransplanted with bone marrow cells (BMC) from healthy donors. Therefore, it is of relevance whether pluripotent hemopoietic stem cells (PSTC) are impaired by the cytotoxic regimen prior to the sampling of the marrow autograft. In long-term bone marrow cultures (LTBMC) in vitro hemopoiesis can be maintained for several weeks. The cells proliferating in these cultures are resistent to the cytotoxic drug 4-Hydroperoxycyclophosphamide which is indicative of cells more immature than restricted or unrestricted progenitor cells, such as GM-CFC and GEMM-CFC. By using our micro LTBMC technique we analyzed the production of GM-CFC in cultures which were initiated with either normal BMC or BMC taken for autologous transplantation from patients in complete remission from acute myeloid leukemia or high grade lymphoma. There was no significant difference in the period of in vitro proliferation when LTBMC of BMC from the above patients were compared to cultures of normal BMC. However, our data so far show a decreased GM-CFC production in cultures initiated with BMC from patients after chemotherapy

H 329 PREINCUBATION WITH RECOMBINANT IL-1 PROVIDES PROTECTION WHILE PREINCUBATION WITH RECOMBINANT IL-6 INCREASES THE IN VITRO TOXICITY OF 4-HYDROPEROXYCYCLOPHOSPHAMIDE TOWARDS EARLY HUMAN PROGENITOR CELLS. Jan Moreb, James R. Zucali and Roy S. Weiner, Department of Medicine, University of Florida, Gainesville, FL 32610
The ability of human recombinant IL-1β to protect hematopoietic progenitor cells from 4-hydroperoxycyclophosphamide (4-HC) was determined by treating bone marrow mononuclear cells (BMMN) with different doses of 4-HC (12.5-150 ug/ml) immediately, following 20 hrs incubation with medium, or after 20 hrs incubation with IL-1β (100 ng/ml). The cells were then cultured in semisolid medium containing IL-3, erythropoietin and 5637 CM. The lethal dose of 4-HC was found to be greater by 50% for BMMN preincubated with medium alone and by 100% for BMMN preincubated with IL-1β when compared to BMMN treated immediately with 4-HC. Medium obtained from BMMN cells preincubated for 20 hrs contained 0.5-1.0 ng/ml IL-1 as measured by ELISA. Using a similar assay system, we have also compared the ability of 20 hrs preincubation with human recombinant IL-6 to protect colony forming cells from high doses of 4-HC. BMMN cells were incubated with IL-6 (240 ng/ml), IL-1β (100 ng/ml) or medium alone for 20 hrs prior to treatment with 4-HC. After washing out the 4-HC, the cells were cultured in semisolid medium as described above. Preincubation with IL-1β resulted in an increased number of different colony types including blast cell colonies in comparison with medium alone. Preincubation with IL-6 resulted in a marked reduction in total colonies, including blast cell colonies, when compared to IL-1β or medium alone. Therefore, our results confirm a role for IL-1β in the protection of early colony forming cells from 4-HC and suggest that IL-6 is not involved in mediating this protective effect.

H 330 EXPRESSION OF THE Evi-1 GENE IN VARIOUS ORGANS AND CELL LINES, Kazuhiro Morishita, Evan Parganas, Diana S. Parker and James N. Ihle, Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, TN, 38101
The Evi-1 locus was initially identified as a common site of viral integration in murine myeloid leukemias, designated as ecotropic viral integration site 1. We have recently demonstrated (Morishita et al, 1988, Cell 54, 831) that retroviral insertions in this locus result in the activation of gene encoding a protein of the zinc finger family. Viral insertions occur in the 5' region of the gene near non-coding exons and activated expression of a 5 kb transcript by promoter insertion. Based on the cDNA sequences, the Evi-1 gene product is a 120 kd protein which contains two domains with seven and three repeats of a DNA binding consensus sequence(zinc finger) initially described in the xenopus transcription factor III A(TFIIIA). To study the normal function of the gene, we have examined the pattern of expression of the Evi-1 gene.
Among a variety of murine tissues, including bone marrow, spleen and fetal liver, transcripts of 5 kb have only been detected in ovary and kidney. Among a variety of myeloid cell lines which have been examined only lines containing rearrangements in the in the Evi-1 locus(DA-1, NFS-58, NFS-60, NFS-78, M1) contained detectable transcripts. Transcripts were not detectable in cell lines representing a variety of other lineages with the exception of the embryonal stem cell line SCC-PSA1. The major transcript in PSA1 cells was approximately 4 kb and this smaller transcript is due to deletion of sequences in the 3' non-coding region and usage of other polyadenylation sites, based on northern analysis and sequencing of cDNA clones. Expression of the Evi-1 in PSA-1 cells was not associated with any detectable rearrangements of the gene. Since PSA1 cells will differentiate when cultured in the absence of feeder layers, we examined the effects of differentiation on expression of the of the Evi-1 gene. Following removal from adherent cells and concomitant with differentation was a loss of transcripts suggesting the possiblility that the Evi-1 gene may be involved in maintaining the de-differentiated state of embryonal stem cells.

H 331 A NOVEL DIRECTIONAL cDNA CLONING METHODOLOGY USING EPSTEIN-BARR VIRUS EPISOMAL EXPRESSION VECTORS, Dwight M. Morrow, Richard K. Groger and Mark L. Tykocinski, Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106
Recent studies from our laboratory and others have established the utility of self-replicating Epstein-Barr virus (EBV) vectors for high level gene expression in human hematopoietic cells. In light of our previous demonstration that the Rous sarcoma virus 3' long terminal repeat (RSV 3' LTR) is well-suited for gene expression in human myeloid and lymphoid leukemia cell lines and nontransformed T cell clones, we have proceeded to generate a series of EBV episomal expression vectors, designated REP, incorporating this promoter. One of these, REP5, has been specifically designed for directional cDNA cloning and expression. In this vector, unique BamHI and HindIII sites are sequentially arrayed downstream of the RSV 3' LTR. An adapter-addition directional cDNA cloning methodology, a variant of a previously described linker-addition method, was developed whereby unique BamHI and HindIII cohesive ends tag the 5' and 3' (polyadenylated) ends, respectively, of cDNA inserts. These inserts are then directionally cartridged into the REP5 vector. An HL-60 cDNA library was generated by this method, and the directionality of cDNA inserts was confirmed by DNA sequencing. In contrast to a recently reported method (Mol Cell Biol 8:2837-2847, 1988) whereby pre-existing cDNAs are secondarily transferred into an EBV episome, our approach entails the cloning of cDNAs directly into an EBV episomal vector in an orientation-specific manner. The availability of EBV episome-based cDNA expression libraries opens up novel possibilities for functionally sorting through unknown genes in human hematopoietic cells.

H 332 REGULATORY ELEMENT OF IL-3 GENE EXPRESSION BY HTLV-I TRANSACTIVATOR $p40^{tax}$, Junji Nishida, Shoichiro Miyatake, Mitsuaki Yoshida*, Ken-ichi Arai and Takashi Yokota, Molecular Biology Departmenr, DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304; *Cancer Institute, Tokyo, Japan. IL-3 is the lymphokine produced by antigen or lectin stimulated T cells. To study the regulation of IL-3 gene expression, plasmids carrying 5' upstream region of human or mouse IL-3 gene and the bacterial CAT gene were introduced transiently in human T cell clone, Jurkat cells. Treatment of Jurkat cells with calcium ionophore/TPA induced endogenous IL-3 mRNA, while the same stimulus did not activate transfected IL-3 gene. Therefore the region covering 4 kb upstream of TATA box may not be sufficient for the induction of IL-3 by calcium ionophore/TPA. We previously reported that $p40^{tax}$ the transactivator encoded by HTLV-I genome, activates IL-3 and other lymphokine genes in the absence of immunological stimulus (Yokota et al., Immunol. Rev., 1988). The regulatory elements of GM-CSF gene was mapped to CLE-1 (conserved lymphokine element-1), CLE-2 plus GC-rich region (MIyatake et al., Mol. Cel. Biol., 1988). In contrast, although IL-3 gene share the similar DNA motif, the regulatory element of IL-3 gene which mediates the response to $p40^{tax}$ was mapped within GC-rich region (5' boundary of the region is at position -76 upstream of the CAP site), which does not show homology with GM-CSF regulatory region. This is different from other conserved sequenses such as CLEs. This region is conserved both in human and mouse, and does not contain NFκB motif which is responsible for the activation of HIV-LTR, SV40 early promoter (Miyatake et al., Mol. Cell. Biol., in press) and IL-2 receptor gene by $p40^{tax}$ or calcium ionophore/TPA (Leung & Nabel, Nature, 1988, Ruben, Science, 1988).

H 333 HEMOPOIETIC POTENTIAL OF CFU-D. E. Niskanen, T. Shellem and V. High, University of Virginia School of Medicine, Charlottesville, VA. Previously we have reported that the hemopoietic precursors called CFU-D can give rise to multipotential CFU-S (day 12) and CFU-MIX. Recently, we have demonstrated that cells derived from CFU-D colonies can also form large (> 0.5 mm) in vitro colonies containing macrophages (HPP-CFC). In this study we assessed the potential of cells from CFU-D colonies to grow in the in vitro CFU-blast assay system. In the presence of EL-4 CM, between 40% and 50% of the resulting colonies contained blasts on day 5 of subculture. Further incubation of these cultures led to transformation to macrophage-, granulocyte-, megakaryocyte- and normooblast-containing colonies. The resulting colonies contained up to 5×10^5 cells. The average diameter of colonies was 6 mm (range 2-10 mm). In another set of experiments, 24 lethally irradiated female mice were each injected with five male-derived CFU-D colonies. Twelve of the mice were long-term survivors and analysis of DNA by Southern blot analysis revealed the presence of Y chromosome derived DNA in the bone marrow, spleen, lymph nodes and thymus. These data suggest that CFU-D has high proliferative potential and capability to reconstitute hemopoietic tissues.

H 334 BIOLOGICAL EFFECTS OF CONSTITUTIVE v-myc EXPRESSION IN HUMAN MONOBLASTIC U-937 CELLS. F. Oberg[1], L.G. Larsson[1], I. Ivhed[1], P. Stockbauer[1], M. Gidlund[2], U. Pettersson[3], B. Vennstrom[4] and K. Nilsson[1], [1]Department of Pathology, Univ. of Uppsala, [2]Karolinska Inst., Stockholm, [3]Dept. of Medical Genetics, Univ. of Uppsala, Sweden, [4]EMBL, Heidelberg, B.R.D.
The human monoblastic cell line U-937 can be induced to differentiate by phorbol ester (TPA) and several physiological inducers. When induced to differentiate U-937 cells will undergo morphological and functional changes similar to those observed in normal monocytic differentiation and eventually become irreversibly growth arrested. Differentiation or serum starvation, resulting in growth inhibition, is associated with a decrease in c-myc expression. The role of c-myc in regulation of proliferation and growth was further studied by introduction of the OKIO, v-myc gene into U-937 cells. U-937 clones, constitutively expressing v-myc, were characterized with respect to growth, phenotype and capacity to differentiate after TPA induction. These cells were shown to be inhibited in the capacity to terminally differentiate. The morphology of the v-myc expressing cells was very similar to the parental cells. However, using a panel of monoclonal antibodies studies revealed a suppression of MHC class I expression in the clones expressing high v-myc levels. This suppression was also demonstrated at the mRNA level. A slighlty higher expression of transferrin receptors was also noted. No v-myc related changes in the surface expression of CD11b, CD13, CD15, CD16, CD33, CD34, CD38, FcRI and FcRII were observed. Other phenotypic changes of the v-myc clones include increased sensitivity to lysis by NK-cells and to TNF induced cytotoxicity. Studies of growth behaviour showed an increased sensitivity to serum factors and a drastic increase in clonogenicity in semi-solid medium. The response of the v-myc clones to several physiological and non-physiological inducers are currently being investigated. The results from these studies will also be presented.

H 335 TRANSFORMATION OF PRE-B CELLS TO IL-7 INDEPENDENCE BY A NON-AUTOCRINE MECHANISM. Robert W. Overell, Karen E. Weisser, and Anthony E. Namen. Immunex Corp., Seattle, WA 98101 USA.

Murine bone marrow cells were infected with Abelson murine leukemia virus (A-MuLV) or ΔRM, a recombinant retrovirus which co-expresses v-Ha-ras and v-myc. When infected cells were grown in the Whitlock-Witte culture system the cultures rapidly overgrew (5-7 days) with pre-B cells, which grew to high saturation densities. These populations were then seeded in agarose in the absence of stromal elements and in the presence and absence of IL-7. Colonies of IL-7 independent pre-B cells arose in the ΔRM- and A-MuLV-infected groups, which could readily be established into cell lines. No IL-7 independent growth occurred in the controls. The frequency of IL-7 independence in the ΔRM-infected cultures was ~1/1,000 seeded cells. Immortal, IL-7 independent clones of ΔRM and A-MuLV pre-B cells expressed the expected retroviral mRNAs. However, no IL-7 mRNA expression could be detected, indicating that IL-7 independence had not occurred by an autocrine mechanism.

H 336 MACROPHAGE RECOGNITION OF UNDIFFERENTIATED MURINE ERYTHROLEUKEMIA CELLS, Charles C. Pak and Isaiah J. Fidler, Department of Cell Biology, M. D. Anderson Cancer Center, Box 173, Houston, TX, 77030. Macrophages are present in the bone marrow as the center of hemopoietic clusters and serve as sites of active erythroid differentiation. We now propose that the interaction of macrophages and erythroid cells can be studied *in vitro* by the use of murine erythroleukemia cells (MELC). MELC are Friend virus transformed erythroid precursor cells that can be induced to differentiate to relatively mature, hemoglobin producing erythroid cells (DIFF) subsequent to a five day incubation in 5 mM hexamethylenebisacetamide. DIFF cells are visually identified by staining with benzidine for the presence of hemoglobin. Light and electron microscopic examinations have revealed that macrophages selectively bind the undifferentiated MELC. Macrophages and MELC form rosettes, with a central macrophage surrounded by undifferentiated MELC. In contrast only few (<5%) of cells from DIFF cultures bind to macrophages. Quantitative binding assays utilizing ^{51}Cr labeled target cells reveals that macrophages bind MELC at levels 7-10 fold higher than DIFF cells. Binding of MELC is independent of serum components but requires the presence of divalent cations. Treating the MELC and macrophages with proteases reduced the extent of specific binding, suggesting the importance of proteins and/or glycoproteins for this interaction. The supernatant of trypsin treated MELC also mediated a reduction in binding between macrophages and MELC. The MELC system provides an opportunity to study the interaction of macrophages with erythroid cells with the advantages of a cultured cell line. The cell-cell interactions that occur in this system may illuminate the role of the macrophage in tissue turnover processes in the bone marrow.

H 337 CYTOCHEMICAL CHARACTERIZATION OF STROMAL CELL POPULATIONS IN LONG TERM B CELL CULTURES. **Greg A. Perry and Darrin J. Anderson, Dept. of Anatomy, University of Nebraska Medical Center, Omaha NE 68105.**

In vitro hematopoiesis is dependent upon the presence of a stromal cell monolayer for the long term maintenence of hematopoietic stem cells. Although the stromal elements associated with Dexter type long term bone marrow cultures (LTBMC) have been relatively well characterized, little attention has been focused on the stromal components found in long term cultures for B lymphocytes (LTLC; Whitlock & Witte, PNAS 79:3608, 1982). Consequently we have used cytochemical stains to characterize the various adherent stromal populations found in LTLC. Adherent monolayers were fixed and stained for Alkaline Phosphatase (Al-P), Acid Phosphatase (Ac-P), Tartrate Resistant Acid Phosphatase (TRAP) and Alpha Napthol Acetate Esterase (ANAE) activity. Many of the cell types found within LTBMC monolayers were also present in LTLC. $ANAE^+$ and $Ac\text{-}P^+$ adherent cells were identified as soon as two days after culture initiation. Cells positive for these cytochemical stains dominated the monolayer for at least the first 10 weeks of culture. $Al\text{-}P^+$ cells were also identified soon after culture initiation (day 3). Such $Al\text{-}P^+$ cells were morphologically identical to the "blanket" cell population found in LTBMC, and were often associated with lymphopoiesis. Finally, a novel cell population of $TRAP^+$, $Ac\text{-}P^+$ large multinucleate cells was identified. Morphologically and cytochemically these cells resembled osteoclasts. Although osteoclasts have been reported in LTBMC of both primate and human marrow, this cell population has not been previously reported in LTLC. This finding may be significant in light of results of Van Slyck et al. (A.J.Med.Sci. 291:347, 1986), who reported two cases of nonsecretory multiple myeloma which produced Osteoclast Activating Factor (i.e. IL-1-β). (Supported by NIH grant AI 25222).

H 338 SIMULTANEOUS MORPHOLOGICAL DETECTION OF ERYTHROPOIETIN GENE EXPRESSION AND INTRACELLULAR ERYTHROPOIETIN FORMATION. Ivan N. Rich, Christina Vogt and Gudrun Noe. Department of Transfusion Medicine, University of Ulm, D-7900 Ulm/Donau, F.R.G.

It is now known that under normal, steady-state conditions, the kidney produces little if any erythropoietin (EPO). For many years now, we have been postulating that the macrophage is not only capable of releasing EPO, but is also capable of modifying EPO release in response to physiological oxygen tensions. In situ hybridization using biotinylated EPO DNA probes, streptavidin-gold to detect, and reflection-contrast microscopy to observe the signal, has shown that 14 day cultured macrophages derived from unseparated and unstimulated mouse bone marrow cells can express the EPO gene, a result substantiated using Northern blotting. Using a monspecific, polyclonal antibody to EPO prepared in our laboratory using affinity chromatography on a recombinant EPO (Behringwerke) column, and immunofluorescence staining, we now demonstrate the simultaneous presence of gene expression and intracellular mature protein formation of erythropoietin in functionally and phenotypically resident macrophages (F4/80+ve). Since about 3% of the macrophages present in normal mouse bone marrow also express the EPO gene, it is possible to conclude that under normal, steady-state conditions, a macrophage subpopulation for the local, microenvironmental production of erythropoietin occurs.

H 339 DETECTION OF MINIMAL DISEASE IN HEMATOPOIETIC MALIGNANCIES OF THE B CELL LINEAGE USING COMPLEMENTARITY DETERMINANT REGION 3 (CDR3)-SPECIFIC PROBES Giovanni Rovera, Masao Yamada, Sheila Hudson, Olivia Tournay, Susan Bittenbender, Sara S. Shane, Beverly Lange, Yoshihide Tsujimoto and Andrew J. Caton, The Wistar Institute of Anatomy and Biology, Philadelphia, PA 19104.

Immunoglobulin heavy chain (IgH) gene assembly that occurs during the commitment of hematopoietic stem cells to the B lineage results in formation of DNA sequences unique for each B cell clone. Since malignancies of the B lineage are of clonal origin, the DNA sequences were utilized to generate probes specific for the malignant clones. Oligonucleotide primers homologous with V_H and J_H segments were used in the polymerase chain reaction (PCR) to amplify and sequence the DNA coding for the complementarity determinant region 3 (CDR3) of leukemic cells. Oligonucleotide probes generated on the basis of the sequencing data were tested for specificity of reactivity against the amplified CDR3 DNA regions of acute lymphoblastic leukemia (ALL) cells and of normal lymphocytes and found to be highly specific for the CDR3 DNA from which the probes were derived.

In mixing experiments a leukemic specific DNA sequence could be detected when the PCR amplified material was cloned into recombinant DNA libraries and screened with specific probes. An accurate quantitation of residual disease in a case of ALL in remission, was made by amplifying and cloning the CDR3 DNA sequences present in his marrow cells followed by screening of the recombinant colonies using a CDR3 probe specific for his leukemic cells.

These studies indicate that it is possible to rapidly identify DNA sequences coding for the CDR3 and generate diagnostic probes specific for leukemic cells of each patient. These diagnostic probes can be used to monitor the extent of minimal disease in B cell lineage malignancies and in other hemopoietic malignancies with rearrangements of the IgH locus.

H 340 GROWTH FACTORS DEPENDENT PHENOTYPE CHANGES OF HUMAN T CELL LEUKEMIA CELL LINES WITH T(8;14) TRANSLOCATIONS, Daniela Santoli, Beverly Lange, Brent Kreider, Janet Finan, Steven C. Clark, Peter Nowell, and Giovanni Rovera, The Wistar Institute, Philadelphia, PA

Two human leukemic cell lines (TALL-101 and TALL-103) were established from children with highly undifferentiated T leukemia with t(8q23; 14q11) translocations. In both cases the leukemic cells did not grow in culture in serum supplemented medium but could be established as permanent cell lines using medium supplemented with rh IL3 and rh GM-CSF. Evidence that the cell lines of T cell lineage was provided by genotypic analysis showing rearrangement of the T cell receptor loci. Establishment of TALL-103 cells was achieved using medium supplemented either with IL3 alone, GM-CSF alone or IL2 alone. Despite the same genotypic characteristics and identical karyotype the three cell lines showed marked phenotypic differences. All cell lines were positive for CD25 (the p55 subunit of the IL2 receptor); whereas cells grown in IL2 were 100% CD3-positive, cells grown in IL3 and GM-CSF were CD3-negative and lacked T cell specific surface markers, but were positive for CD15, a myelomonocytic antigen. Cells grown in GM-CSF showed a marked tendency to differentiate into adherent, non proliferating and phagocytic monocytic-like cells. These findings indicate that 1) a subset of undifferentiated and clinically very aggressive T leukemia in childhood is characterized by a t(8q23; 14q11) translocation, 2) the leukemic cells can be consistently (2/2 cases) established as cell lines in culture using recombinant growth factors and 3) the phenotype of the cells in culture is strongly influenced by the growth factors present in the environment. This last finding suggests that this particular subset of childhood T cell leukemia has biphenotypic (T-myeloid) potential.

H 341 **THE CELL CYCLE STATUS OF MURINE SPLEEN COLONY FORMING UNITS (CFU) HARVESTED FROM THE ADHERENT LAYER OF LONG TERM MARROW CULTURES.** J.G. Sharp, D.A. Crouse, S.L. Mann & S.S. Joshi. Univ. Neb. Med. Center, Omaha, NE 68105.

When the adherent cells of long term marrow cultures are harvested and injected intravenously (IV) as a single cell suspension into lethally irradiated recipients, approximately equal numbers of day 8 (D8), day 12 (D12) or day 14 (D14) spleen colonies are formed. In contrast, if the harvested cells are maintained short term (up to 6 hours) as organ cultures (OC) and transplanted under the kidney capsule, few if any D8 spleen colonies are formed but expected or greater numbers of D12 and D14 colonies are observed. This has lead us to suggest that maintaining stem cell/stromal cell contacts in the OC preserves the hierarchically more primitive D12 or D14 CFU and inhibits their differentiation. However, Blackett (Leuk. Res. 11:365, 1987) has suggested that the timing of colony formation has no hierarchical implications and simply reflects a feedback mechanism potentially originating from maturing granulocytes which acts only on proliferating stem cells. Thus, less responsive, non-proliferating stem cells would form colonies later. Consequently, we have determined the hydroxyurea sensitivity of the colony forming cells in the circumstances described above. When the adherent cells are injected IV the proportion of D8 CFU killed was 40%, D12, 22% and D14, 0%. When cultured and transplanted as OC, there were no D8 CFU to assay and hydroxyurea did not kill any D12 or D14 CFU. This result suggests that the contact of stem cells with stroma inhibits cycling of the stem cells. Also, these results appear compatible with Blackett's hypothesis. This in turn implies that cycle associated cell surface molecules may have an important role in stem cell regulation. Indeed, when the surface of bone marrow cells was non-cytolytically modified with 2.5% n-butanol, the number of D8 and D12 colonies was increased by 234% and 152% respectively. Whether this results from increased cycling, altered f-factor or redistribution of the injected stem cells and whether this is inhibited by granulocytes is under investigation.

H 342 HEMOREGULATORY PEPTIDE-DIMER CAN RESCUE MICE FROM LETHAL DOSES OF CYCLOPHOSPHAMIDE AND IRRADIATION, Ove V.Solesvik, Nils Pape and Dan Longo, NCI-BRMP, FCRF, Frederick, MD 21701. *Hemoregulatory Peptide monomer (HPm) is a peptapeptide first found in extracts from human granulocytes. The synthetic version pGlu-Glu-Asp-Cys-Lys (NYCOMED AS,Oslo) has shown inhibitory activity on murine and human myeloid progenitor cells (Laerum 1984). In contrast the homodimer (HPd), coupled by a disulfide bridge between the two cysteine residues, has proven to have stimulatory activity on myeloid progenitors (Laerum 1988). A single iv injection of 1.2 ng/kg HPd to healthy mice produced a transient increase (100% on day 2) of bone marrow CFU-C. Continuous infusion over seven days resulted in a longer lasting increase of CFU-C numbers, but the effect vanished rapidly after the infusion ended. The potential of HPd as a immunorestorative agent has been studied in normal and tumor-bearing mice after sublethal and lethal doses of cyclophosphamide (CTX). HPd treatment two days prior to the insult produced detrimental effects, whereas HPd given post-CTX generated dramatic beneficial effects, including accelerated PBL recovery and increased survival. The known hematopoietic growth factors IL-1 (pre CTX) and CSF-gm (post CTX) were used as positive controls. They showed significant increase in survival, but both proved to be significant inferior to HPd. Furthermore, HPd administered after lethal doses of irradiation to normal mice could rescue 70% of the animals compared to 90% by IL-1 and 20-30% by CSF-gm.*

Ref. O.D. Laerum and W. Paukovits (1984) Exp.Hematology 12:7-17
O.D. Laerum et al.(1988) Exp. Hematology 16:274-280

H 343 RETROVIRAL INFECTION OF ENRICHED STEM CELLS FROM MURINE BONE MARROW

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We have achieved over 100 fold enrichment of 12 day spleen colony forming cells (CFU-s) from murine bone marrow. The separation is achieved by fluoresence activated flow sorting for Thy 1^{low} Mac 1^-, Gr 1^-, $B220^-$, $CD4^-$, $CD8^-$, Ly 1^- cells[1,2]. Enriched cells were co-cultivated with a high titer, $5x10^6$ CFU/ml, neo virus producer cell line, then injected into lethally irradiated animals. The spleen colonies present at 12 days were excised and the DNA probed for integrated proviruses. Many colonies contained one or more proviral integrations. Thus, enriched stem cells can be infected with reasonable efficiency. Various numbers of enriched stem cells were injected into lethally irradiated animals. By 4 months, 50% of animals that received 500 cells are alive, and 80% of animals that received 1000 cells are alive. No animals receiving 250 cells survived. Surviving animals were tested at 2 months post transplant and all were >90% donor repopulated, using a donor specific DNA probe on peripheral blood DNA. The long term reconstituting stem cell is therefore co-enriched with CFU-s using this method. We are now attempting to infect enriched long term reconstituting stem cells. Using unique proviral integrations as markers, we intend to study the clonality of reconstitution in this simplified system. In addition, we are also attempting to infect the stem cells with retroviral vectors expressing exogenous growth factor receptors or oncogenes in order to test the effects of various growth signals on the differentiation and proliferation of these cells in vitro and in vivo.

References

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H 344 EXPRESSION OF MURINE AND HUMAN INTERLEUKIN 5 (IL5) IN EUKARYOTIC SYSTEMS,

Tavernier J., Devos R., Van der Heyden J., Hauquier G., Bauden R., Kawashima E., and Fiers W., Roche Research Gent, J. Plateaustraat 22 B-9000 Gent, Belgium.

The gene coding for murine IL5 was isolated from TPA-induced EL4-ExC5 cells. The amino acid sequence was found to be identical to the published sequence, with the exception of an Arg→His replacement at position 79. A human IL5 gene was chemically synthesized allowing the introduction of strategically located restriction enzyme cleavage sites. Both these genes have been expressed in various eukaryotic systems.

Using SP6 polymerase generated transcripts, up to 1 ug.ml-1 of biologically active IL5 can be secreted after injection in Xenopus laevis oocytes. In the presence of 35S- labeled amino acids, IL5 can be labeled in this system to high specific activity. In the case of murine IL5, deletion of the 3' untranslated region causes a 10 fold increase of the secreted product. This same phenomenon was observed in NIH-3T3 cells using the pBMG-Neo vector . A correct in frame fusion of the coding region for mature human IL5 to the α -mating type factor leader sequence of Saccharomyces cerevisiae was constructed . Mature Il5 was constitutively secreted into the yeast medium and was purified to homogeneity. In all cases mentioned above, recombinant IL5 was found to be glycosylated and the biological activity was dependent on a 40-50 Kd dimer structure. No biological activity could be correlated with the monomer (20-25 Kd) obtained after reduction. Deglycosylation did not affect the biological activity. Purified recombinant human IL5 was 125 I- labelled and used in receptor binding studies. rhIL5 has specific activities of appr. 1-2.10E3 U.mg -1 in the BCl-1 assay and of at least 5.10E6 U.mg-1 in a bone marrow eosinophil peroxidase assay.

H 345 IDENTIFICATION OF GENES UNIQUELY EXPRESSED IN MOUSE PLASMACYTOMA CELLS,

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Mouse plasmacytoma cell lines differ from a mature, immunoglobulin secreting murine B cell line (A20.2J) by several notable features: 1) morphology, 2) lack of expression of certain B cell markers (e.g. surface Ia antigen), 3) lack of endogenous c-myc mRNA expression, and 4) the consistent dominance of the plasmacytoma phenotype in somatic cell hybrids formed with all other types of lymphoid cells. To identify genes which distinguish the plasmacytoma from the closely related B cell lymphoma, we have prepared a subtractive cDNA library. Incorporation of PCR (polymerase chain reaction) technology at several steps has facilitated the construction and analysis of this library. Thus far, we have identified 15 genes which are expressed differentially in both the parental and an unrelated plasmacytoma compared to the subtractive B cell partner. We are continuing to define the extent of difference in gene expression in these cell lines and will soon begin to characterize the genes that are differentially expressed. Our long term goal is to identify and isolate genes which determine or are specific for the phenotype of terminally differentiated plasma cells.

H 346 USE OF EPISOMAL VECTORS FOR GENE EXPRESSION IN HUMAN BONE MARROW STROMAL CELLS,
Matthew C. Weber, Kenichi Harigaya* and Mark L. Tykocinski, Institute of Pathology, Case Western Reserve University, Cleveland, OH 44106 and *Department of Pathology, Chiba University School of Medicine, Chiba 280, Japan.
Human bone marrow stromal cells are essential for the maintenance of hematopoietic stem cell proliferation and differentiation in long-term bone marrow cultures. To molecularly probe the complexities of stromal cell:stem cell and stromal cell:leukemic cell interactions, we have developed stable gene transfer capabilities for these cell types. Studies were undertaken to optimize transfection efficiencies for an SV40 large T-immortalized human marrow stromal cell line, KM-102. We achieved stable transfection of Epstein-Barr virus (EBV) episomal replicons into KM-102 cells using three alternative transfection modalities: electroporation, lipofection and scrape-loading. Using our previously reported set of EBV episomal replicons [PNAS(USA) 85:1040, 1988] in which one of five eukaryotic promoters directs transcription of the prokaryotic chloramphenicol acetyltransferase gene, we established that the Rous sarcoma virus 3' long terminal repeat (LTR) and the SV40 early promoter function well in human stromal cells. In contrast, the lymphopapilloma virus 5' LTR showed little activity. The inducible rat GRP78 and human metallothionein II_A gene promoters exhibited high basal activity with modest increases in activity when induced with the calcium ionophore A23187 and Cd^{++}, respectively. This demonstration of efficient transfection and stable gene expression in human stromal cells using EBV episomal vectors, along with our previous similar findings for human leukemic cells using the same vectors, will now permit the molecular dissection of stromal cell:stem (or leukemic) cell interactions using gene transfection.

H 347 A NEW IN VITRO COLONY ASSAY FOR HUMAN MULTIPOTENT PROGENITOR CELLS,
Neil Wilkie[1], Gunther Konwalinka[2], Eric Wright[3] and Ian Pragnell[1]; [1]Beatson Institute for Cancer Research, Switchback Road, Bearsden, Glasgow G61 1BD, Scotland; [2]Department of Internal Medicine, University of Innsbruck, Anichstrasse 35, 6020 Innsbruck, Austria; [3]LRF Radiation Leukaemogenesis Laboratory, MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD, U.K.
We have developed an in vitro colony assay for human multipotent progenitor cells which detects a high proportion of the primitive progenitor compartment. Mononuclear cells from bone marrow are plated at low concentration in semi solid medium containing recombinant GM-CSF, a source of human CSF-1 and 25% foetal calf serum. These culture conditions support the formation of macroscopic multilineage colonies at an average incidence of 135 ± 85 per 10^5 mononuclear cells. The colony forming cells (CFU-A, colony forming unit, type A) give rise to colonies with marked heterogeneity which contain 1-2 x 10^4 cells per colony, suggesting a high proliferative potential. The CFU-A have a variable cycling status, and those with the highest proliferative potential have the lowest proportion in cycle. Developing colonies replate with a high efficiency (50-75%) and 3% give rise to secondary CFU-A. Cells with similar properties are also detected in peripheral blood at a ten to twenty fold lower incidence. Comparison of human CFU-A to our previously reported results on murine CFU-A (Pragnell, et al., 1988, Blood, 72, 196) strongly suggest that the assay detects a high proportion of the primitive progenitor compartment, in contrast to previously reported assays for human multipotent progenitor cells (Leary and Ogawa, 1987, Blood, 69, 953; Ash,Detrick and Zanjani, 1981, Blood, 58, 309).

In Vivo and Clinical Studies

H 400 KINETICS OF HUMAN HEMOPOIETIC CELLS FOLLOWING IN VIVO ADMINISTRATION OF GM-CSF.
Massimo Aglietta, Wanda Piacibello, Fiorella Sanavio, Alessandra Stacchini, Franco Apra', Marina Schena, Carlo Mossetti, Clara Monzeglio, Felice Gavosto. Clinica Medica A, Dip. Scienze Biomediche ed Oncologia Umana and Div. Ginecologia, Osp. St. Anna. Via Genova 3, 10126 TORINO, Italy.
The kinetic changes induced by GM-CSF on hemopoietic cells were assessed in physiological conditions by administering GM-CSF (Sandoz, Basel, 8 ug/Kg/day) for 3 days to 9 patients with solid tumors and normal bone marrow (BM) prior to chemotherapy. GM-CSF increased the number of circulating granulocytes and monocytes; platelets, erythrocytes, lymphocyte number and their subsets were unmodified. GM-CSF increased the percentage of BM S phase BFU-E (from 32+7% to 79+16%), day 14 CFU-GM (from 43+20% to 82+11%) and day 7 CFU-GM (from 41+14% to 56+20%). The percentage of BM myeloblasts, promyelocytes and myelocytes in S phase increased from 26+14% to 41+6%, that of erythroblasts from 25+12% to 30+12%. This suggests that GM-CSF activates both erythroid and granulopomonopoietic progenitors but that, among the morphologically recognizable precursors, only the granulo-monopoietic lineage is a direct target of the molecule. GM-CSF increased the birth rate of BM cells from 1.3 to 3.4 cells/h and decreased the duration of the S phase from 14.3 to 9.1 h and the cell cycle time from 86 to 26 h. After treatment discontinuation, the number of circulating granulocytes and monocytes rapidly fell. The proportion of S phase BM cells dropped to values lower than pretreatment levels, suggesting a period of refractoriness to antineoplastic agents.

H 401 DIFFERENTIAL ACTIVATION OF DOG, HUMAN AND MONKEY PERIPHERAL BLOOD GRANULOCYTES BY RECOMBINANT HUMAN GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR, IN VIVO HEMATOPOIETIC ACTIVITY IN DOGS. Charles Lam, Peter Mayer, Jürgen Besemer, Dieter Dennig[§] and Walter Knapp[§]. Sandoz Forschungs Institute and [§]Institute of Immunology, Vienna, Austria.
While it is extensively documented that human granulocyte-macrophage colony-stimulating factor (GM-CSF) controls the production and functional activity of human granulocytes and macrophages, relatively little is known about its effects on cells obtained from other species. The molecular cloning of the complementary DNA for human GM-CSF has made it possible to determine the cross reactivity of the purified recombinant human material (rh GM-CSF) on cells of other species.The results presented herein show that specific receptors for human GM-CSF exist on bone marrow cells and mature circulating dog granulocytes. The number of the receptors and the apparent binding affinity (K_D) of the rh GM-CSF to its receptors on granulocytes were similar to that observed either on human or monkey cells. In agar cultures of dog bone marrow cells, rh GM-CSF induced colony formation in a dose dependent manner. The effect of rh GM-CSF on the respiratory burst in mature dog granulocytes was less dramatic compared to human or monkey cells. Whereas human and monkey cells responded to amounts as low as 20pg per ml rh GM-CSF avidly by producing oxygen metabolites, the dog granulocytes were stimulated significantly only with concentrations between 2 - 20 ng per ml. In vivo, daily s.c.administration of the rh GM-CSF (50 or 150 ug/kg) in dogs over seven days induced a prompt 2-3 fold rise in the counts of circulating white blood cells of which the neutrophils were the most predominant. The induced leukocytosis was, however, lower than that previously observed in monkeys. Taken together, the results suggest that human GM-CSF does not appear to exhibit absolutespecies-specificity.

H 402 RhGM-CSF AND RhG-CSF PROMOTE EARLY LEUKOCYTOSIS AND NEUTROPHILIA IN THE NEWBORN RAT. M.S. Cairo, C. VandeVen, K. Kommareddy, K. Sheikh, D. Mauss and H. Modanlou. Childrens Hospital of Orange County, Miller Childrens Hospital Long Beach, U.C. Irvine, Orange CA 92668. RhGM-CSF and rhG-CSF, two widely studied hematopoietic growth factors, have been demonstrated to induce proliferation, maturation, and release of myeloid stem cells and mature PMNs from human and animal **adult** bone marrows. Reduced bone marrow progenitor cells, neutrophil storage pool (NSP) depletion and peripheral neutropenia are characteristic of human and animal **newborn** bone marrows. We investigated the effect of administering intraperitoneal rhGM-CSF and rhG-CSF to Sprague-Dawley newborn rats (< 24hr). NB rats treated with I.P. CSF demonstrated significant leukocytosis and neutrophilia at 6 and 24 hrs. (1.0, 3.0, 5.0 mg/kg) WBC (10^3) at 6 hr: 3.0 mcg/kg rhGM-CSF vs control (8.0 $\pm$ 0.5 vs 4.3 $\pm$ 0.9) $p < .003$; 24 hr; (7.7 $\pm$ 1.7 vs 3.8 $\pm$ 0.2) $p < .008$; WBC (10^3) at 6 hr rhG-CSF vs control: 3.0 mcg/kg, (6.6 $\pm$ 1.2 vs 4.3 $\pm$ 0.1) $p < .03$; 24 hrs; (8.1 $\pm$ 0.2 vs 3.75 $\pm$ 0.2) $p < .003$. The ANC was also significantly elevated at 6 hrs following I.P. CSF. RhGm-CSF vs control-6hr; (3.0 mcg/kg) 1827 $\pm$ 25 vs 379 $\pm$ 10 ($p < .001$), rhG-CSF vs control-6hr; 1698 $\pm$ 40 vs 371 $\pm$ 10.1 ($p < .001$): Additionally, femurs were surgically removed and flushed with buffer and marrow NSP (PMN + Band + Meta) determined at 6 and 30 hrs following rhGM-CSF (3.0 mcg/kg vs control) 6hrs: NSP 5.2 $\pm$ 1.3% vs 16 $\pm$ 2.4% $p < .001$; confirming that peripheral neutrophilia at 6hr was secondary to NSP egress from the bone marrow. At 30hr NSP and control demonstrated no difference. We postulate that CSF may promote early leukocytosis and neutrophilia in the newborn rat by inducing NSP egress from the bone marrow, but do not exhaust NSP reserves.

H 403 ACUTE IN VIVO HEMATOLOGIC EFFECTS OF RECOMBINANT HUMAN IL-3 AND IL-6, INDIVIDUALLY AND IN COMBINATION, ON THE BONE MARROW AND CIRCULATION OF THE RAT, del Castillo, Juan and Ulich, Thomas R., Department of Pathology, University of California, Irvine, CA 92717.

IL-3 administered intravenously (IV) as a single injection induces peripheral neutrophilia and monocytosis beginning at 4 to 6 hours, peaking at 8 hours, and subsiding to normal by 12 to 24 hours. IL-3-induced peripheral neutrophilia at 8 hours is accompanied by a decrease in mature marrow neutrophils, by a left-shifted myeloid hyperplasia, and by an increase in pronormoblasts. IL-6 administered as a single IV injection induces a biphasic neutrophilia with a peak at 1.5 hours and a sustained wave of neutrophilia between 4 and 12 hours, a mild lymphocytosis at 0.5 hours and a mild lymphopenia between 1.5 and 4 hours, and a reticulocytosis between 12 and 24 hours. The bone marrow did not show a decrease in neutrophils at 1.5 hours (suggesting that the initial neutrophilia is due to demargination), but did at 12 hours demonstrate a mild left-shifted myeloid hyperplasia and a tremendous erythroid hyperplasia of intermediate and late normoblasts. The combined IV injection of IL-3 and IL-6 induced a synergistic peripheral neutrophilia, a striking left-shifted myeloid hyperplasia, a marked erythroid hyperplasia, and a dramatic increase in marrow mast cells that was not induced by either IL-3 or IL-6 alone.

H 404 THE EFFECT OF LEWIS LUNG TUMOR (LLca) GROWTH ON THE SYNERGISTIC ENHANCEMENT OF CFU-M BY IL-1 + M-CSF. M.J. Evans and C.J. Kovacs. Division of Radiation Biology & Oncology, East Carolina University School of Medicine, Greenville, NC 27858.

During the growth of the LLca, both the erythroid (BFU-E) and granulomonocytic (CFU-M, -GM, -G) progenitor compartments are progressive expanded (Cell Tissue Kinet 18: 235, 1985; Exp. Hemat. 14: 165, 1986). We have more recently observed that the 12d CFU-S, in addition to the previously reported 8d CFU-S, and the CFU-GEMM stem cell subpopulations (HSC-SP's) are also expanded during tumor growth. Because both IL-1 and M-CSF are required for the in vitro stimulation of a HSC-SP which gives rise to a high proliferative potential progenitor of the CFU-M, studies were designed to determine whether the effect of the LLca tumor on the CFU-M compartment of the marrow could be simulated in vitro by these "synergizing" cytokines. Preincubation of the marrow from normal (non-tumor bearing) mice with optimal colony-forming doses of M-CSF was found to enhance the number of CFU-M. Under identical conditions, preincubation carried out with increasing dose levels of IL-1 (0-10^3 U per ml), in the absence of M-CSF, failed to stimulate colony formation. When CSF was combined with IL-1, however, a synergistic enhancement of M-CFU was observed. Conversely, similar studies carried out using the marrow from tumor-bearing animals demonstrated that the synergistic ability of IL-1 + M-CSF observed in the normal marrow was progressively diminished with increasing tumor burden. Collectively, these data suggest that tumor growth results in either (a) an alteration of the age distribution of the IL-1 + M-CSF responsive HSC-SP, or (b) the progressive appearance a suppressor cell that is functionally operative in vitro but whose activity is restricted in vivo.

H 405 EVIDENCE THAT GRANULOCYTE-COLONY STIMULATING FACTOR (G-CSF) IS NEUTROPOIETIN. W.P. Hammond, A.R. Canin,* E. Csiba,* L.M. Souza,* D.C. Dale. Univ. of Washington, Seattle, WA, *AMGen, Thousand Oaks, CA.

Two normal mongrel dogs were treated with recombinant human G-CSF (rh G-CSF) by daily s.c. injection at 10μg/kg/d for 30 days. Daily blood counts, weekly sera for antibody titers, and bone marrow aspirates prior to, during, and after treatment were obtained. Initial responses consisted of an acute rise in neutrophil counts, peaking within 6 hours of injection (17,000/μl), followed by a sustained elevation in daily pre-injection neutrophil counts, with peaks between 40 and 45,000/μl during the first 25 days. Platelet and reticulocyte counts did not change during the treatment period. Lymphocyte and monocyte counts showed mild, inconsistent elevations during treatment. After day 27, a profound decrease in neutrophil counts occurred (100-400/μl) which persisted for 5 months. Hematocrit, platelet, monocyte and lymphocyte counts remained normal during this prolonged neutropenic period. An associated rise in antibody titer to rh G-CSF from 1:40 pretreatment to >1:1000 at day 30 was noted (dog #1). Marrow aspirations revealed granulocytic hyperplasia during treatment and hypoplasia during neutropenia. Initial neutrophilia, followed by severe neutropenia and marrow hypoplasia, with the associated elevation in antibody titers to rh G-CSF suggests that G-CSF is the physiologic neutropoietin.

H 406 CYTOKINE PROTECTIVE PROPERTIES AND THE HEMATOPOIETIC STEM CELL SUBPOPULATIONS (HSC-SP's): DRUG AND RADIATION STUDIES. C.J. Kovacs, M.J. Evans, J.M. Gooya, J.P. Harrell and K.M. McGowen. Division of Radiation Biology & Oncology, East Carolina University School of Medicine, Greenville, NC 27858.

Because of the apparent role of specific cytokines in hematopoietic regulation, we have initiated studies to determine whether the hematopoietic system can be preconditioned ("Primed") to reduce the the selective sensitivities of individual HSC-SP's to specific chemotherapeutic agents as previously reported by this laboratory (NCI Monog., 5: 45, 1987) and by so doing, provide protection to the hematopoietic system. Protection was evaluated by the following parameters: (a) animal survival, (b) temporal recovery kinetics; and (c) cell survival. Using IL-1 at doses of $1x10^3$ U (low) or $2.5x10^5$ U (high) per mouse, administered at either 12 or 24 hr prior to drug treatment, low dose IL-1 was found to provide enhanced and modest protection against 5-FU (150mg/kg) and Cytoxan (250mg/kg), respectively, but little effect against the damage resulting from either Adr (10mg/kg) or DDP (8mg/kg). Conversely, at the higher dose of IL-1, protection was realized against each of the agents as well as ionizing radiation. These preliminary observations suggest that the effectiveness of IL-1 as a protective agent may be related to the drug sensitivities of the individual HSC-SP's which appear to distinguish between the acute, delayed and residual damage associated with specific chemotherapeutic agents. Comparisons between the effect of IL-1 on the hematopoietic and gastrointestinal system will be presented.

H 407 IN VIVO EFFECTS OF RECOMBINANT HUMAN INTERLEUKIN 3 ALONE OR IN VARIOUS COMBINATIONS WITH OTHER CYTOKINES AS GM-CSF, G-CSF AND EPO IN NORMAL CYNOMOLGUS MONKEYS, Dorothee Krumwieh and Friedrich R. Seiler, Research Laboratories of Behringwerke AG, P.O. Box 1140, 3550 Marburg/Lahn, West Germany

With the availability of recombinant human hematopoietic growth factors like GM-CSF, G-CSF, IL-3 and EPO in large amounts, it has become possible that clinical studies with the single factors have been started in a variety of partial or complete bone marrow failures like aplastic anemia, congenital agranulocytoses, chemotherapy induced pancytopenia, myelodysplastic syndromes, chronic infections with neutropenia or early stages after bone marrow transplantations. However, it could be demonstrated already in vitro that highly synergistic activity is one of the major effects if the factors are acting together. We have treated healthy cynomolgus monkeys in order to define the role of IL-3 in long-term and short-term application followed either by GM-CSF/G-CSF or by EPO adminsitration. Long-term application of IL-3 (100 µg/kg/d x 30) induced a pronounced increase in basophils (10-fold), platelets and lymphocytes. If a short-time application (2d) of IL-3 is used in combination with GM-CSF, only a raise in platelets was to be seen. The prolongation of the IL-3 administration period (8d) followed by GM-CSF then resulted in a highly synergistic effect with an additional raise of neutrophils, eosinophils and monocytes. Combination studies were performed with suboptimal concentrations of GM-CSF and G-CSF (10 µg/kg/d). If the IL-3 treatment was followed by EPO administration (10 µg/kg/d), an increase was observed in hematocrit and hemoglobin values due to synergistic action of IL-3 and EPO with respect to erythroid progenitors.

H 408 DETECTION OF REGULAR OSCILLATIONS IN SERUM GRANULOCYTE - COLONY STIMULATING FACTOR LEVELS IN CYCLIC HEMATOPOIETIC DOGS, Clinton D. Lothrop, Jr., David J. Warren, Maryanne Maniatis, Malcolm A.S. Moore, and J. B. Jones, Univ. of Tenn., Knoxville, TN 37901 and Sloan-Kettering Cancer Center, New York, NY 10021.

Cyclic hematopoiesis (CH) of gray collie dogs and some humans with childhood onset CH is a genetic disease of the hematopoietic stem cells. The hallmark of this disease is regular fluctuations of peripheral blood cells with cycles of 12 to 14 days in dogs and 20 to 24 days in humans. Neutrophils cycle more prominently than other hematopoietic cells in affected humans and dogs. The hematopoietic hormones erythropoietin, thrombopoietin and colony stimulating activity (CSA) are known to cycle in CH dogs. The CSA has been shown to cycle inversely with neutrophil cycles in both human and dogs with CH. These studies were carried out prior to characterization of the major hematopoietic growth factors so that the exact molecular form of the CSA was not known. We have recently shown that administration of recombinant human granulocyte colony stimulating factor (G-CSF) to CH dogs eliminates the cycles of neutropenia. This suggested that G-CSF might be the cyclic CSA in CH dogs. Therefore, we measured the serum G-CSF level with a NFS-60 cell proliferation assay in 5 cyclic hematopoietic (CH) and 2 normal dogs. Serum G-CSF was <2 units/ml in the normal dogs and the CH dogs during the recovery phase of the cycle. During the neutropenic phase of the cycle the serum G-CSF level was increased up to 500 units/ml in the CH dogs. The 3 to 4 day peak of G-CSF activity occurred just prior to neutrophil recovery at 14 day intervals in all 5 CH dogs. The addition of anti-G-CSF completely neutralized the G-CSF activity in CH dog serum. The studies suggest that G-CSF is the cyclic CSA in CH dogs and point to the importance of G-CSF in regulating neutrophil production.

H 409 RECOVERY OF IMMUNE REACTIVITY IS MASKED BY NATURAL SUPPRESSOR (NS) ACTIVITY WHICH IS INDUCED DURING HEMATOPOIETIC RECOVERY FOLLOWING CYTOREDUCTIVE PROCEDURES, Tom Maier, Dept. of Microbiol./Immunol., U. Colorado Sch. Med., Denver, CO 80262

In both humans and animals there is an extended period of immunoincompetence which follows high dose cytoreductive treatments either themselves or with bone marrow transplants. For example, mice injected with 250 mg/kg cyclophosphamide (CY) become completely unresponsive in vitro in mitogen and mixed leukocyte reaction cultures. This complete anergy lasts about 10-14 days and the animals do not regain normal responses until 3-4 weeks after CY treatment. This immunoincompetence is one of the major drawbacks to the efficacious use of cytoreductive cancer therapies. The current thinking in regard to this cytoreductive-induced immunoincompetence is that the cytoreductive treatment has killed the immunocompetent cells and that it takes a certain amount of time for the immune system to repopulate with the right combination of mature cells to again mount a productive immune response. However, at lease some cytoreductive procedures also induce potent NS cell activity, which appears inversely related to immune reactivity. This NS cell activity peaks during the hematopoietic recovery phase after these high dose cytoreductive treatments. The NS cell's inhibitory activity may be actually prolonging the period of immunoincompetence past that caused by the direct damage to the immune system. Therefore, this period of immunoincompetence may be dramatically shortened if the suppression caused by the NS cells could be decreased. We have begun experiments which show this is the case — immunocompetence does return sooner than is realized after cytoreductive treatments, but is in fact masked by the NS cell activity.

H 410 SINGLE DAILY SUBCUTANEOUS ADMINISTRATION OF rhGM-CSF REDUCES HEMATOPOIETIC TOXICITY OF CHEMOTHERAPY, Roland Mertelsmann, Margitt Wieser, Gregor Schulz*, Albrecht Lindemann, Wolfgang Oster and Friedhelm Herrmann, Department of Hematology, University of Mainz, D-6500 Mainz, FRG, *Behringwerke, D-3550 Marburg, FRG

Myelosuppression is a major cause of chemotherapy-associated morbidity and often the limiting factor for both, dose of chemotherapeutic drugs as well as the frequency of treatment. We investigated whether single daily subcutaneous (s.c.) administration of rh Granulocyte/Macrophage CSF (rhGM-CSF) could reduce the period of neutropenia and neutropenia-related infectious complications in patients receiving intensive chemotherapy for various hematologic malignancies and solid tumors. The first course of treatment consisted of chemotherapy only. Twenty-five patients developing neutropenia as a result of the first cycle of chemotherapy, were elegible to receive the next cycle of the same chemotherapy followed by s.c. administration of daily rhGM-CSF (250 mcg/m²). The nadir of neutrophil counts was less pronounced and shorter in duration in all courses with GM-CSF including 3 patients being autotransplanted twice for testicular cancer. Courses without rhGM-CSF were complicated by infections in 80% of patients requiring hospitalization. In contrast, patients on GM-CSF experienced infections episodes in 10% only. These findings demonstrate (1) that daily s.c. rhGM-CSF is efficacious in patients with neutropenia secondary to a broad spectrum of chemotherapeutic protocols and (2) that it can be administered safely to outpatients reducing morbidity as well hospital costs for inpatient care. In patients with refractory or relapsed NHL we are currently investigating whether chemotherapeutic doses can be increased when combined with rhGM-CSF resulting in higher CR rates.

H 411 THERAPEUTIC ADMINISTRATION OF RECOMBINANT HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR ACCELERATES HEMOPOIETIC REGENERATION AND ENHANCES SURVIVAL FOLLOWING RADIATION INDUCED MYELOSUPPRESSION. M. L. Patchen, T.J. MacVittie, B.D. Solberg and L.M. Souza, Armed Forces Radiobiology Research Institute, Bethesda, MD and AMGen, Thousand Oaks, CA.

Introduction: The primary cause of death following radiation injury or intensive radiotherapy is infection resulting from radiation-induced myelosuppression. Granulocyte Colony-Stimulating Factor (G-CSF) stimulates granulocyte proliferation and differentiation. Because granulocytes are important for host defense against postirradiation infections, G-CSF was evaluated in irradiated mice for the ability to 1) accelerate hemopoietic regeneration, and 2) enhance survival. **Methods**: C3H/HeN mice received cobalt-60 radiation on day 0 and G-CSF (2.5 ug/day s.c.) or saline on days 3-12 postexposure. Bone marrow (BM) and splenic (SPL) cellularity and granulocyte-macrophage progenitor cell (GM-CFC) recoveries were evaluated in mice exposed to 6.5 Gy. Mice exposed to 8 Gy were evaluated for pluripotent hemopoietic stem cell recovery (using endogenous spleen colony-forming units; E-CFU-s) and survival enhancement. **Results**: As early as day 10 postirradiation significant increases in cellularity and GM-CFC were observed in G-CSF mice. By day 17 BM and SPL cellularity in G-CSF mice, respectively, were $3.18+0.14\ 10^6$ and $187.08+7.29\ 10^6$ compared to $1.75+0.13\ 10^6$ and $89.21+5.62\ 10^6$ in saline mice. BM and SPL GM-CFC in G-CSF mice at this time, respectively, were 1835+140 and 41889+4471 compared to 353 + 49 and 2547+405 in saline mice. By day 20, peripheral WBC, PMN, and PLT counts were also elevated in G-CSF mice. G-CSF mice exposed to 8 Gy exhibited both increased E-CFU-s (3.6+0.4 vs 1.3+0.4) and increased survival (55% vs 30%). Initiating G-CSF at day 1 (instead of day 3) postexposure further increased E-CFU-s (8.4+2.3) and survival (70%). **Conclusions**: These results demonstrate that therapeutic G-CSF 1) accelerates hemopoietic regeneration following radiation-induce myelosuppression 2) enhances survival following potentially lethal irradiation, and 3) is most effective when initiated within 1 day postexposure.

H 412 THE HEMOREGULATORY PEPTIDE pGluGluAspCysLys AUGMENTS HEMOPOIETIC RECOVERY AFTER CYTOSTATIC DRUG TREATMENT OF MICE. W.R.Paukovits[1], K.Binder[1], J.Paukovits[1], M.Guigon[2], G.Furtmüller[1], O.Laerum[3]

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Peptides: The hemoregulatory peptide (HP5b) monomer was synthesized by a novel solid phase strategy designed for optimal protection of the thiol group against oxidation. The primary synthetic product, with the thiol protecting group still attached, can easily be purified and was activated in situ prior to application. The disulfide bonded dimer of HP5b was prepared from ACM-protected HP5b by oxidation with iodine and purified by column chromatography.

Results: In vitro the monomeric peptide inhibits (10^{-12} M) CFU-GM colony formation, whereas the dimeric form is a potent enhancer (10^{-15} M) of GM-CSF induced colony formation. In vivo the monomer prevents the recruitment of CFU-S (induced by injecting 900 mg/kg ara-C into mice) into proliferation by injecting 0.6µg HP5b (10^{-9} moles) at -2,2,and 6 hrs relative to the ara-C application. HP5b does however not reduce the growth of splenic CFU-S nodules when injected into irradiated hosts after bone marrow transplantation. Multiple high dose ara-C applications (4x900 mg/kg) result in the death of 90% of the mice within 7-9 days. Reconstitution of the hemopoietic system by a bone marrow transplant decreases the mortality to about 45%. A single injection of HP5b (0.6µg at 26 hrs, when few CFU-S proliferate) decreases the mortality to 58%, not significantly different from the transplanted groups. The dimeric stimulatory form of HP5b (1.4 µg/kg/day) showed a strong accelerating effect on the recovery of hematopoiesis after combined ara-C/nitrogen mustard treatment. HP5b monomer did not change the ara-C sensitivity of transformed cell lines, even not in such cases where it had a direct effect on cellular proliferation (e.g. HL-60). It did not induce differentiation of HL-60 or Friend cells.

These results suggest that the chemically synthesized HP5b monomer and dimer may be useful as myeloprotectors in cancer chemotherapy.

H 413 AMPLIFICATION OF THE HEMATOPOIETIC EFFECTS OF HUMAN INTERLEUKIN-1ß (IL1ß) IN INTACT MICE FOLLOWING BLOCKADE OF PROSTAGLANDIN BIOSYNTHESIS, Louis M. Pelus, Department of Hematopoietic Regulation, Sloan Kettering Institute, New York, NY. 10021
Given the ability of IL1 to stimulate Prostaglandin E2 synthesis, which induces myelopoietic suppressor cells in intact mice, we investigated the effects of recombinant IL1ß on myelopoiesis in mice treated with Indomethacin (Indo), a prostaglandin biosynthesis inhibitor. Single i.v. injection of 0.15-2.4 ug/mouse IL1ß resulted in induction of bone marrow (BM) and spleen (SP) cells suppressing normal CFU-GM by 42-49%, and a 15-30% reduction in total BM and SP CFU-GM within 6 hrs. Suppressor cells were observed at 6, 18 and 24 hrs after a single injection of 0.04 ug IL1ß, with loss of effect by 48 hrs. Readministration of IL1 resulted in reinduction of the suppressor cells with identical kinetics. Pretreatment of mice with Indo (100 ug, s.c.) 24 hrs prior to IL1 resulted in the inability to detect this suppressive mechanism and significant enhancement of BM and SP CFU-GM. Enhancement of total BM CFU-GM by 75-80% was observed at 48-72 hrs post IL1ß, while total SP CFU-GM was increased 275-500% in the 24-72 hrs post injection in IL1 plus Indo treated mice. Administration of 0.04 ug/day IL1ß for 4 days to Indo treated mice resulted in no detectable suppressor cells, a 15X increase in SP CFU-GM and a 6X increase in PB absolute neutrophil counts (ANC) at 4-7 days post IL1ß. In mice given IL1ß alone, a 4X increase in SP CFU-GM and a 3X increase in ANC were observed on day 7. These results indicate that IL1ß induces a myelopoietic suppressor mechanism which can be blocked by inhibition of prostaglandin synthesis. As a consequence, significant stimulatory effects of IL1ß on myelopoiesis are masked. These findings have considerable implication to clinical trials of human IL1ß.

H 414 CSF-1 AND IL-1α CONCENTRATION IN THE SERUM OF ACUTE LEUKEMIA AND BONE MARROW TRANSPLANTED PATIENTS DURING THE INITIAL POST TREATMENT PERIOD, V. PRALORAN[1,2]; N. MILPIED[1] E.R. STANLEY,[2] Department of Hematology, CHRU NANTES, 44035, FRANCE[1]; and Department of Cell Biology, Albert Einstein College of Medecine, BRONX, NY 10461.[2]
The role of hematopoïetic growth factors CSF-1 (M-CSF) and IL-1α for in vitro proliferation and differentiation of hematopoïetic progenitors is largely investigated. Conversely, there are few reports concerning the biological function, steady state and kinetics of serum levels of these two molecules, during the course of hematological diseases and treatment induced modifications of the hematopoïetic system. We recently developed radioimmunoassays for CSF-1 and IL-1α , detecting these molecules in serum in a range of sensitivity from 30 pg to 12 ng for CSF-1, and from 20pg to 2 ng for IL-1α . We previously reported increased Serum CSF-1 concentrations in myeloproliferative diseases (BLOOD, 1987, 70 Suppl. : 135 a). In this report, we present results from three different groups of patients. Group 1 : acute leukemia patients undergoing an intensive chemotherapy. Group 2 : patients in complete remission of an acute leukemia, and submitted to a conditioning regimen of chemotherapy plus total body irradiation before bone marrow transplantation. Group 3 : patients developing complications during or after the bone marrow engraftment period. Patients from groups 1 and 2 were studied prospectively from day 0 to day 30 or 40 of treatment. Patients from group 3 were studied rectrospectively using freezed stored samples. Data concerning the CSF-1 and IL-1α modifications are presented and compared to the clinical course of these patients.

H 415 **ANTI-IL-5 ANTIBODY INHIBITS HELMINTH INDUCED EOSINOPHILIA IN VIVO**, Donna M. Rennick, Susan Hudak, John Jackson, Brian Seymour, Robert L. Coffman, Department of Immunology, DNAX Research Institute of Cellular and Molecular Biology, Palo Alto, CA 94304. Anti-mouse IL-5 monoclonal antibodies have been used to directly assess the role of IL-5 in parasite-related eosinophilia. When mice were injected with larvae of Nippostrongylus brasiliensis, they developed 1) a peripheral blood eosinophilia, 2) large numbers of eosinophils, granulocytes, and mononuclear cells in the lung, and 3) elevated numbers of IL-5-responsive eosinophil colony forming cells (Eo-CFC) in bone marrow. In contrast, parasitized mice infused with anti-IL-5 antibodies did not develop a peripheral blood eosinophilia, their lung infiltrates contained only a few eosinophils, and their bone marrow contained a lower number of IL-5-responsive Eo-CFC. These results show that IL-5 is the major lymphokine regulating the production of mature peripheral eosinophils in this disease state.

H 416 **RADIATION CHIMERAS ENGRAFTED WITH BONE MARROW FROM ALLOPHENIC MICE REVEAL SELECTIVE GENOTYPIC CONTRIBUTIONS TO HEMATOPOIESIS.**

Gary Van Zant, Brigid P. Holland, Paul W. Eldridge and Jau-Jiin Chen, Department of Cell Biology and Anatomy, Texas Tech University Health Sciences Center, Lubbock, TX 79430. We have previously described two phenotypic differences relevant to hematopoiesis in DBA/2 and C57BL/6 inbred mouse strains (Van Zant *et al.*, Cell, 35:639, 1983): (a) 24% of CFU-S in DBA/2 mice are killed by hydroxyurea whereas only about 3% of C57BL/6 CFU-S are killed by similar treatment, thus suggesting that a higher rate of proliferation and stem cell turnover may be characteristic of the DBA/2 strain; (b) in allophenic (chimeric) mice made by combining DBA/2 and C57BL/6 embryos, the genotype of RBCs is skewed toward DBA/2 and, conversely, the genotype of circulating lymphocytes is skewed toward C57BL/6, thus suggesting that genotype-limited differences exist in differentiation along different hematopoietic lineages. Results reported here are based on studies of hematopoiesis in radiation chimeras made by transplanting C57BL/6<-->DBA/2 allophenic marrow into lethally irradiated B6D2F$_1$ hosts. These experiments were designed to examine the relative repopulating potential of stem cells of the two genotypes in a competitive situation. Several of our experiments have suggested that hybrid resistance to marrow engraftment is absent in this combination of donor and host. Using electrophoretic variants of glucose phosphate isomerase to quantitatively distinguish between donor (C57BL/6 and DBA/2) and host (B6D2F$_1$) blood cell genotypes, we have studied hematopoiesis for over a year in these mice. Analysis of red and white blood cells obtained from serial bleedings of host mice show that during the first 1-6 months after engraftment the percentage of blood cells of DBA/2 genotype is significantly (20-50%) higher than the percentage of marrow cells of DBA/2 origin in the marrow transplant used to establish the radiation chimera. After about six months, the relative contributions of DBA/2 and C57BL/6 stem cells to blood cell genotype is the same as the blood and marrow cells of the donor allophenic mouse. Similarly, the skewing of red cells toward the DBA/2 genotype is re-established in the blood of the radiation chimeras. These results suggest that DBA/2 stem cells have an initial competitive advantage during repopulation, possibly owing to a higher stem cell proliferation. However, after several months, hematopoiesis in the radiation chimeras very closely parallels the genotypes of blood and marrow cells in the donor mouse.